

INVESTIGATIONS INTO
DIFFERENTIATION

AND OTHER MORPHOLOGICAL CHANGES
IN MALIGNANT TUMOURS
FOLLOWING THERAPEUTIC IRRADIATION
WITH X-RAYS AND RADIUM

BY

S. RY ANDERSEN

With 28 Photomicrographs



EINAR MUNKSGAARD

COPENHAGEN 1949

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Translated from Danish by

Anna la Cour,
née Claessen.

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TREATISE FOR
THE DOCTORATE AT THE UNIVERSITY
OF COPENHAGEN

"Mice and men"



EINAR MUNKSGAARD
COPENHAGEN 1949

*Denne afhandling
er af det lægevidenskabelige fakultet
ved Københavns Universitet
antaget til offentligt at forsvares for den
medicinske doktorgrad.*

København, den 21. april 1949.

*J. Engelbreth-Holm,
h. a. dec.*

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Dedicated
to my wife, the opera singer
MONNA RY ANDERSEN
née Sjøreen



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Preface.

The investigations to be reported in this volume were commenced in 1943 at the University Institute of Pathological Anatomy, Copenhagen and at the Radium Centre in Copenhagen under the influence of Professor *J. Engelbreth-Holm*, M. D. to whom my sincere thanks are due for his unfailing interest in my work and numerous inspiring conversations.

Jens Nielsen, Chief of the Radium Centre, has afforded me ideal working conditions, and with his extensive experience in radiotherapy he afforded me great help for which I want to express my gratitude.

The irradiation experiments were carried out at the Radium Centre, and I want to thank the nurses in the X-ray Room for the care and precision with which the radiations were carried out.

The histological specimens were prepared partly in the Pathological Laboratory of *F. Bang*, M. D. at the Radium Centre, partly at the University Institute of Pathological Anatomy by Miss *H. Rydahl*. I ask Dr. *Bang*, his staff, and Miss *Rydahl* to accept my thanks.

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Last, but not least, I want to thank my wife, *Monna Ry Andersen*, who assisted me in all the animal experiments and who has been untiring in encouraging me and urging me on while I was preparing the manuscript.

8, Harsdorffsvej,
Copenhagen,
December 1948.

S. RY ANDERSEN.

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In addition, a protocol of the experiments (78 pages) will be lodged at the University Institute of Pathological Anatomy, Copenhagen, the Radium Centre in Copenhagen, and the University Library, Department II, Copenhagen, where it can be inspected.

Introduction.

A reduced capacity for differentiation or absence of such capacity is one of the most outstanding features in the morphology of malignant growths and is by many considered to be *the* characteristic of cancer. During the half century while X-rays and radium have been available in the treatment of cancer, the question has often been raised as to whether the irradiations had the power to produce increased differentiation in malignant tumours. The reports of such investigations have been sporadic and the results conflicting.

In view of the theoretical and practical interest which attaches to this problem, I set myself the task of studying it on a broad basis. A number of characteristic and theoretically well-suited types of tumours were examined histologically following irradiation with X-rays or radium, partly therapeutic irradiation of malignant tumours in man by X-rays or radium, partly X-radiation of transplantable mouse tumours. In the latter case the technique and dosage were as far as possible according to the principles generally used in the radiological treatment of cancer. It is generally accepted that the effect of X-rays and radium in therapeutic doses is in principle identical, and therefore the histological reactions will be considered together.

After reviewing earlier investigations I shall report my own studies.

CHAPTER I

On Differentiation.

In this volume the term differentiation is used to designate transformation from a more unripe to a more ripe, specialized state. Differentiation is always attended with organization of cells. Differentiation and absence of differentiation correspond to the concept of prosoplasia and anaplasia introduced by *Hansemann* in 1890. Differentiation (= prosoplasia) thus indicates a more specialized function of the cells. As early as 1897 *Hansemann* pointed out that it was not in actual fact the task of morphological investigations to elucidate the function of the cell, but that the kind or stage of its activity were so often expressed in its shape that the latter afforded a basis for conclusions.

An outstanding feature of malignancy — although it is not demonstrable in all cases — is a more or less diminished or abnormal differentiation. It is so characteristic that it constitutes one of the most important clues in deciding histologically whether a specimen is cancer or not. Similarly, the absence of differentiation is often an important criterion of the major or minor malignancy of a growth. Accordingly, it is very important to establish the grade of differentiation, and numerous workers have set up systems for this end.

Broders, in 1920, graded the differentiation on the basis of 537 cases of carcinoma of the lip. The system has later been modified slightly and now comprises 4 grades (*Broders*, 1940).

Grade 1: Tumours with about 100—75 per cent of the cells entirely or partly differentiated and about 0—25 per cent. more or less undifferentiated.

Grade 2: About 75—50 per cent. of the cells differentiated, the remainder undifferentiated.

Grade 3: About 50—25 per cent. differentiated, the remainder undifferentiated.

Grade 4: About 25—0 per cent. differentiated, the remainder undifferentiated.

Broders does not count the individual cells, but allocates the tumours to the individual grades on the basis of an estimate of the number of differentiated and undifferentiated cells. Whether a cell is considered differentiated or undifferentiated depends largely on the tissue from which it is derived. The important thing is the difference in differentiation between the normal tissue from which the tumour has arisen and the tumour cells. It has been emphasized by *Broders* that the number of mitotic figures (particularly abnormal ones) plays an important rôle in the estimation.

Broders' system has proved of value in the histological classification of e. g. squamous-cell carcinoma of the skin, adenocarcinoma of the rectum and fibrosarcoma in which the histological signs of differentiation are easily recognizable. It is less valuable in the classification of mammary carcinoma, and for instance in the estimation of the very characteristic malignant melanomas it has proved practically worthless. Basal-cell carcinoma of the skin constitutes a separate group, not directly comparable with squamous-cell carcinoma. (In *Broders'* opinion (1932) the cells in a basal-cell carcinoma of the skin are highly differentiated (Grade 1), the difference in differentiation between them and the basal epidermal cell from which they are considered to arise, however, being only slight).

Broders' grading is based on the principle that "cancer" is growth of more or less undifferentiated cells with the capacity to regenerate, whereas fully differentiated cells, e. g. in an epithelial pearl, have irrevocably lost their capacity to divide and thereby their malignant properties (*Broders* 1921).

According to *Broders* the grade is usually the same throughout the tumour, and after a wide study of the subject he has arrived at the result that the grade is a fitting expression of the degree of malignancy, the most highly differentiated tumours being considered least malignant. This must, however, in my opinion be taken with some reservation, as one must bear in mind that there are two kinds of malignancy, biological as well as clinical. *Biological malignancy* depends solely on the properties of the tumour, *inter alia* largely on its differentiation. When all other factors are equal, an undifferentiated tumour should therefore be more malignant than a more highly differentiated one arising from the same tissue. However, the degree of differentiation alone is probably not the only factor deciding the biological malignancy. Other, partly unknown factors in the tumour must be considered. For instance, it is well-known that certain carcinomas of the thyroid gland are so highly differentiated in spite of marked biological (and clinical) malignancy, that the tumour cells are difficult to discern histologically from the normal thyroid cells.

Clinical malignancy, on the other hand, besides being dependent on the biological malignancy, is connected with many other factors: the anatomical site of the tumour, its spread when treatment is instituted, operability and radio-sensitivity, the patient's age and general condition, and lastly with the choice of treatment and the skill and technical resources at the disposal of the physician. Consequently, the clinical malignancy will often differ from the biological malignancy estimated by histological examination, and therefore it is not warrantable to determine the degree of clinical malignancy and the prognosis on the basis of the biological malignancy alone, as has been done by many workers.

Other classifications of the differentiation have been proposed on

lines similar to *Broders'* by *Martzloff* (1923), *Greenough* (1925), *Hueper* (1928 and 1929), *Healy* (1930), *Warren* (1931), *Gates & Warren* (1934), *Warren, Meigs, Severance & Jaffe* (1939), and *Bang* (1941). Some of these systems are, however, concerned with the degree of biological malignancy which then comes to include the degree of differentiation along with other factors like e. g. polymorphism.

It is generally agreed that parakeratosis and cornification in a squamous-cell carcinoma of the skin or a carcinoma of the cervix uteri express a higher differentiation, and that the degree of parakeratosis and cornification aptly express the grade of differentiation, but as pointed out by *Stewart* (1932) the degree of cornification in a tumour must be estimated according to its site. Similarly, glandular formation, e. g. in a mammary carcinoma is considered to mean higher differentiation, which by some is considered to be represented also by "secretion bodies", whereas the amount of stroma is not attributed with importance in this connection (*Greenough* (1925)).

It is often stated that one biopsy specimen from a tumour usually represents the differentiation of the tumour fairly well (*Lahm* (1927), *Hueper* (1929), *Broders* (1932)) but this is contested by several workers (*Hellwig* (1932), *Konzelmann* (1936), *Gulik* (1939), and *Martzloff* (1928)).

The last-mentioned author has examined biopsies from 70 cases of carcinoma of the cervix in which the determination of the histological type has been followed by histological examination of numerous sections from the subsequently removed uterus. In about one-third of the cases the dominant cell type in the tumour could not be definitely established by biopsy, and in 13 per cent. the appearance of the biopsy had been directly misleading. As regards cornification, the examination had failed in 7 of 15 cases, in 6 of them because cornification had not been demonstrable in the biopsy, whereas it had been present in the tumour. The estimate of mitotic figures, giant cells, and nucleoli, on the other hand, had been correct in nearly all cases. According to *Glücksman & Spear* (1945) biopsies removed from the edge of a tumour are of a uniform appearance, unlike specimens from other parts of the tumour.

Polymorphism cannot be included in one's considerations when determining the degree of differentiation, as it is often independent of the latter. Highly cornified and consequently highly differentiated squamous-cell carcinoma of the oral cavity may, for instance, exhibit marked polymorphism. Hyperchromatism and enlargement of the cell nuclei or nucleoli cannot either illustrate the degree of differentiation, whereas the number of mitotic figures must be granted a certain significance, as a cell undergoing mitotic division is undifferentiated. (However, the number of mitoses depends largely on the kind of tissue). Abnormal mitoses *per se*, on the other hand, do not concern the degree of differentiation, although they are often present in particularly large numbers in undifferentiated tumours.

CHAPTER II

Earlier Investigations into Morphological Cell Changes in Malignant Tumours with Special Reference to Differentiation Following Therapeutic Radiation with X-rays and Radium.

(1) A Few Radiobiological Studies.

The biological effect of X-rays and radium is far from having been fully elucidated. As to details the reader is referred to *Worning* (1937 and 1939), and *Ellinger's* monograph from 1941, and as to its physico-chemical basis to *Thygesen* (1944). Most workers have found that the ionizing effect of X-rays as well as radium on the tissues is independent of the wavelength, and as emphasized by *Warren* (1942, 2) a variation in the wavelength, at any rate within therapeutic limits, does not result in qualitative histological difference. The change in the histological reaction which may be entailed by an alteration of the time factor is, according to *Thygesen*, presumably due to the normal rhythmic reactions in the tissues (e.g. mitotic cell division and changes in the osmotic pressure within the cells), whereas the regeneration occurring after radiation injuries is only regarded of limited importance in this respect. According to the modern interpretation, X-rays and radium may be considered to have an identical effect (*Englmann* (1934), *Holthusen* (1933), *Warren* (1944)).

Very little is known about what takes place in a cell following radiation with X-rays or radium. There will always be some time interval before the effect of the radiation is demonstrable histologically or chemically, and this latent period is no doubt an expression of a number of processes elicited by the ionization, factors which we are unable to observe with our present technique. It has been suggested by *Denstad* (1946) that the morphological changes demonstrable in the cells are of chemical nature and he assumes that there is a direct relationship between the ionization of the tissue due to the radiation and the presumed chemical processes. The rays are presumed to damage vital parts of the cell, probably by denaturation of the protein

molecules. All these factors are, however, not yet elucidated as emphasized by *Failla* (1940).

The enlargement of the cells which almost constantly is observed after heavy radiation must be due to increased imbibition of water by the cell, presumably after an intracellular increase in the osmotic pressure (previously demonstrated *inter alia* by *Ewing* (1926)). According to *Failla* this increase in osmotic pressure is not explicable solely by the increase in the concentration of ions and molecules taking place after radiation. On the other hand, it is possible that an increase in the permeability of the cell membrane and a formation of hydrophilic colloids may play a rôle in addition to the increase in ion concentration. But the nucleus is also swollen after radiation and is often as dark as before, and therefore *Failla* assumes that a protoplasmic synthesis also takes place. Swelling of the cells after radiation cannot occur without extra-cellular material, and this may therefore perhaps explain the significance of circulation and diffusion in the tumour and thereby also the nature of the surrounding medium.

It has often been discussed whether radiation could act as a stimulation or whether its effect was purely destructive. Most workers consider the latter explanation to be correct, a view which also is accepted by *Crowther* (1945). In *Packard's* opinion (1931) radiation cannot act as a stimulation in the sense of favourable acceleration in the rate of growth or increase in function. Increased size of the cells following radiation is not normal growth, but injury. According to *Packard* everything points in the direction that radiation does not stimulate cell activity, but is always primarily an injury. Degeneration products after radiation may thus temporarily quicken the tempo of some normal processes (protoplasmic streaming, mitoses, etc.) but are always followed by injury and are not considered as true stimulation.

It is difficult to say whether the nucleus or protoplasm are attacked first, and it is emphasized by *Packard* that the demonstration of changes of one part of the cell earlier than in another is no proof that this part is more severely affected than the others. Neither can we estimate the degree of injury from the appearance of the cell at a particular time. *Lea*, in his comprehensive monograph on the biological actions of radiations (1946), states that when a cell is killed as a result of therapeutic irradiation, cell-death does not occur at once, but at the time of or after the next division which the cell undergoes. Immediate cell-death can only be effected by doses far larger than those used for therapeutic purposes. *Thygesen* (1944) (like *Englmann*, 1938) is of opinion that the destruction of the cancer cells occurs via "the late effect" (chromosomal disturbances), not by direct cell-death. The abnormal mitoses met with after radiation are considered to represent lethal chromosome mutations. "Persisting cells", i. e. individual cells which are able to survive heavy radiation and constitute the starting point of renewed neoplastic growth, presumably represent a phase during which the nuclear apparatus is practically immune against the action of radiation.

(2) Earlier Histological Investigations.

So far, the investigations made in this field have given conflicting results, one group of workers maintaining that the histological radiation reactions in cancer cells represent degeneration and another group stating that the tumour cells differentiate — ripen — during the radiation.

Perthes, in 1903, was the first to give a detailed description of the histological reaction of malignant tumours to radiation (X-rays). He observed degeneration of the cells in carcinoma of the skin and of the mammary gland, blurred cell outlines, protoplasmic vacuoles, and proliferation of the connective-tissue stroma. The action of radiation was considered to be due to degeneration: differentiation is not mentioned.

The first histological studies on animals were made by *Apolant* (1904) who transplanted a solid carcinoma into mice. The tumours were radiated for 10—20 minutes daily, 20 mg. of radium being applied locally for a period up to 15 days. One mouse was killed every day. The first changes observed were proliferation of the connective tissue and an increase in the number of wandering cells. At the outset the tumour cells revealed ill-defined cell outlines and somewhat later a decrease in the number of mitotic figures. Later again there was vacuolar protoplasmic degeneration and the volume of the cells and nuclei was steadily increasing. Some of the swollen tumour cells were undergoing mitosis. In the deep layers where the effect of radiation was less marked, there were chronic inflammatory changes and connective-tissue proliferation. In *Apolant's* opinion the chief effect of radiation is a direct, primary, specific damage to the cancer cells which die and disappear.

Later, the observations made by *Perthes* and *Apolant* have been supported by numerous workers.

In a Danish thesis from 1909 *Meisen Westergaard* described experiments with X-radiation of *Jensen's* mouse sarcoma and human spindle-cell and lymphosarcomas. He reported only a few and rather uncharacteristic changes in the tumour cells, and presumably the rays used were too soft.

Donaldson & Canti (1923) studied 50 cases of squamous-cell carcinoma of the uterine cervix following radium treatment (133—173 mg. for 8 to 24 hours). During the first post-radiation days there were abnormal mitoses, necrosis and enlargement of the cells. From the 7th day there were no mitoses, but increasing fibrosis; they often observed pronounced degenerative changes of the cells which had large, hyperchromatic nuclei. Differentiation is not mentioned.

Kok & Vorlaender (1923), after delivering a single dose of X-rays to mouse carcinomas, found break-down of the tumour cells and proliferation of the connective tissue. They regarded the main element in the tumour destruction to be due to a general effect of the rays on the body, secondarily giving rise to a reaction in the connective tissue and the cells of the tumour. In a paper from 1924 *Vorlaender* reported the same degenerative changes in the tumour cells following radiation of the tumour-bed only.

In two fundamental papers from 1923 and 1924 *Alberti & Politzer* described the behaviour of the mitoses following X-radiation of the corneae of salamander larvae. They observed 3 stages: (1) Primary effect (pyknotic mitoses and a reduction in the number of mitotic figures), (2) interval without mitoses, (3) secondary effect (numerous abnormal mitoses). The primary effect and the interval without mitoses were considered to be due to injury to the chromatin, whereas the secondary effect should concern the factors determining the regular movements of the chromosomes during mitosis. In a monograph from 1934 on the pathology of mitoses *Politzer* established that the results of the experiments with salamander corneae correspond to the findings in malignant growths.

Other workers have further elucidated the changes in mitosis following radiation (*Juul & Kemp* (1930), *Lea* (1938), *Luther* (1940 and 1943), and *Jüngling & Langendorff* (1941)). The admirable investigations made by *Alberti & Politzer* have been confirmed in all essentials.

Changes in mitosis cannot throw any light on the differentiation following radiation, and will therefore not be dealt with further. The same applies to the behaviour of the centrioles after radiation, studied by *Fogg & Warren* in 1941 and 1942, the mitochondria, and the Golgi apparatus, dealt with by the same authors in two papers from 1937. Consequently, the behaviour of these structures following radiation is considered of no interest in connection with this work.

Berezanskij (1928) and *Horn* (1928) found only non-specific degenerative changes in tumours following radiation with X-rays and radium.

Holthusen (1933) also regarded the cell changes in the tumours to be of a purely degenerative nature and rejected the possibility of an effect stimulating the functional activity.

Englmann, in 1938, gave an excellent and sober survey of radiation changes in human carcinomas (and normal tissue) following therapeutic X-rays and radium.

A suitably fractionated X-radiation only prevents the mechanism of cell division in the tumour, whereas other activities, especially the tendency to growth, are preserved, though somewhat affected, and this results in pathological enlargement of the cell. The irritant to cell division increases with the enlargement of the cell, and at last the "division pressure" gets the better of the daily dose of radiation, and the cell divides. The number of mitotic figures in the tumour increases rapidly as soon as the cell has reached a certain size. The mitoses are so abnormal, that division cannot be completed, and cell-death occurs now, in the first place because the daily, small dose of radiation is lethal in this susceptible phase, and in the second place because the resting cell nucleus is so far affected by the daily doses, that the mitosis cannot be accomplished, even though the radiation is perhaps discontinued before mitosis sets in. If division of the abnormal pluripolar mitoses into several nuclei is accomplished in spite of all, protoplasmic division is bound to fail. The result is giant cells with several nuclei. If division is suppressed altogether, the cells die, exhibiting ever increasing volume and vacuolation of the nuclei.

Englmann has followed the percentage of mitoses in a tumour treated with fractionated X-radiation (100 r daily for 3 weeks) and drawn a mitosis curve. The total number of mitotic figures steadily decreased as the

enlargement of the cells advanced. Towards the end of the treatment there was a sudden, steep increase in the number of mitotic figures which reached a number far exceeding what had been observed before the treatment. A week after the treatment was concluded, the number of mitotic figures again decreased rapidly. Those observed during treatment were pathological, in numbers increasing to about 100 per cent. after the lapse of a week.

In *Englmann's* opinion the changes in the nuclear substance are mutations of lethal nature. Still, he interprets these changes as well as those of the protoplasm as non-specific degenerative processes, emphasizing that the same changes may be observed in spontaneous break-down of the tumour and following other actions. He does not mention the possibility of differentiation after radiation.

Melnick & Bachem (1937) studied transplantable rat tumours following X-radiation, (1) massive treatment (185—2960 r), (2) fractionated treatments (46, 92, or 185 r daily from 4—30 times, i. e. a total dose of 370—5550 r), (3) modified protracted-fractionated treatments, (4) saturation treatment. The kilovoltage was 120 and the intensity 185 r per minute.

In *Flechner-Jobling rat carcinoma* (an undifferentiated squamous-cell carcinoma without the slightest hint of cornification) they found (in a total of 32 tumours) enlargement of the cell, abnormal mitoses, and calcified nuclei. Nothing is stated about differentiation following radiation. In *Jensen's rat sarcoma* (a spindle-cell sarcoma without collagen fibres) they found similar changes in a total of about 10 tumours). The *Walker rat tumour* (a carcinosarcoma), on the other hand, was found to be very radio-resistant, exhibiting slight, uncharacteristic cell changes in merely 1 of 8 cases.

In *Melnick & Bachem's* opinion the sensitive cells undergo primary degeneration of the nature of simple necrosis with nuclear pyknosis and karyorrhexis, whereas refractory tumour cells are altered in a specific manner after radiation by way of a radical change in their chromosomal structure (a mutation) into a race of abnormal cells (hyperchromatic giant cells) which, after a period of proliferation, perish and calcify.

These authors do not either mention the possibility of differentiation after radiation. Each of their experimental series comprises only a few tumours, and biopsies were removed at very long intervals. The squamous-cell carcinoma mentioned was completely undifferentiated and perhaps without any capacity to differentiate.

Halley & Melnick (1940) report similar changes in mammary carcinomas following pre-operative X-radiation. Differentiation is not mentioned.

Dealing with the condition seen after fractionated X-radiation *Ewing* (1938) does not only attach importance to the direct destructive effect of the rays on the tumour cells, but also to an indirect action on the tumour-bed. Through a "process of adaptation" the latter should be able to acquire radio-resistance, unlike the tumour cells, and perhaps regenerate, even though radiation is continued (this applies both to connective tissue and normal epithelium covering the tumour). In certain circumstances the tumour cells should undergo the same adaptation and become radio-resistant.

Ewing does not mention whether the "adapted" radio-resistant cells which he has observed showed altered differentiation or not, and on the whole the report is somewhat vague.

It has been emphasized by *Worning* (1937) that radiation stops the growth of the undifferentiated cells, whereas the more differentiated ones fail to be affected and continue to develop. *Worning's* experimental objects were the back hairs of guinea pigs. *Chydenius* (1940), after pre-operative radium treatment in 30 cases of carcinoma of the uterine cervix, compared a biopsy specimen from the untreated tumour with 8—10 sections from each operation specimen. He found degenerative changes, but does not touch upon the question of differentiation.

Lacassagne & Gricouroff (1941), in a comprehensive paper on the action of radiations on tissue, have classified the histological changes as follows:

(1) *Immediate death.*

- (a) *Coagulation necrosis* (applies to all tissues subjected to intensive radiation. Absorption is slow, as the circulation of blood stops).
- (b) *Cytolysis* (liquefaction necrosis). This is the way in which the most sensitive tissues perish; the nucleus undergoes pyknosis and bursts (karyorrhexis). Cells less rich in chromatin, but very sensitive, also undergo pyknosis, but not rhexis; the cytoplasm becomes granular and disappears in 3—4 hours.

(2) *Slow death* (= mort différée). After a certain latent period there will be:

- (a) *Arrest of cell division.* The cell grows and at last degenerates.
- (b) *Abortive anomalies in the cell division:*
 - Interruption of the mitosis for 24 hours.
 - Later abnormal mitoses — necrobiosis without cell division predominates.
 - Later still, all the tumour cells disappear.
- (c) *Latent lesions of tardive manifestation* (necrobiosis may be observed several years later).

These workers do not mention differentiation, and the cellular changes described by them are not substantiated.

Reuterwall (1941), after histological examination of a number of operation specimens from mammary cancers, partly untreated, partly submitted to pre-operative X-radiation, reported that he could discern 4 zones in an untreated breast cancer:

- (1) Zone of invasion.
- (2) Zone of cell accumulation.
- (3) Zone of regression.
- (4) Central scirrhous, cicatricial zone.

He states that large untreated tumours often exhibit a scirrhous centre, indicating relative cure, perhaps in part due to nutritive disturbances, but presumably also to processes of immunization against neoplastic growth.

Following radiation he reports that frequently there are lively processes of healing in the form of pronounced degenerative changes in the cancer cells.

Zones (1) and (2) are often narrow, whereas the zone of regression and the central scirrhous, cicatricial zone are increased, containing few or no cancer cells.

Differentiation is not mentioned, but the material is not applicable to decide whether this occurs, as no biopsies were removed before and during X-radiation.

Lenz, in 1942, reported degenerative changes in 38 cases of mammary carcinoma submitted to pre-operative X-radiation, but did not mention differentiation.

Hoffmeyer, in 1943, found changes similar to those described by *Reuterwall* in 50 operation specimens from mammary carcinomas submitted to pre-operative X-radiation (1000—4800 r) and operation 1—11 weeks later.

As his material does not contain biopsies from untreated tumours or biopsies removed in the course of the treatment, it is inapplicable for the elucidation of alterations in differentiation.

Dominici & Barcat, in 1907, were the first to advance the theory of the differentiating (ripening) effect of X-rays and radium on neoplastic cells. They maintained that cure effected by radiation was not due to a destruction of the tumour cells, but to an alteration in the development of the cells in the form of increased ripening (= differentiation). Their first experiments were radium treatment of human polymorphous-cell sarcomas which are stated to have been transformed into angiomatous myxomas by transformation of polymorphous sarcoma cells into fusiform, fixed connective-tissue cells. In later papers (1908, 1 and 2) they state that following radium treatment sarcoma is transformed into fibroma after having temporarily resembled myxomatous tissue, whereas epitheliomas are transformed into benign papillomas. The latter phenomenon may be observed morphologically as a "maturation cornée", the cells going through the various developmental phases after the radiation and ending in actual cornification.

According to *Dominici* (1908) radiation with X-rays is presumably of the same effect as radium. He mentions a maxillary sarcoma which was transformed into a fibroma after radium therapy, 'part of the protoplasm being transformed into connective-tissue fibrils, whereas the remainder of the protoplasm and the nuclei became fixed fibroma cells. The nuclei diminished and shrunk, and the fibroma ended as a connective-tissue scar. Certain epitheliomas were partly transformed into papillomas, others into adenomas. In 1909 *Dominici* established that the "evolutive effect" of X-rays and radium without a doubt applies to all tumours, although it cannot be as easily observed in e. g. epitheliomas as in sarcomas.

These papers are accompanied by drawings, but no photographs, so it is difficult to evaluate the results.

In my opinion *Dominici & Barcat* have presumably mistaken surface epithelium showing the effects of radiation for cancer cells and the normal connective-tissue proliferation following upon radiation for sarcoma cells.

Dominici & Barcat's observations have been supported by a number of workers who also are of opinion that radiation entails increased differentiation in malignant tumours:

Clunet (1910), studying 19 human epitheliomas following X-radiation, found (1) a latent phase, (2) monstrous ripening, characterized by hypertrophy of the nucleus and protoplasm, numerous atypical mitotic figures, and monstrous, multiple, chromophilic nuclei and pseudoparasitic bodies in the cells. Later, he observed (3) cornification, partly of individual cells and partly in the form of epithelial pearls. At times he found atypical cornification unpreceded by the normal preliminary stages. (4) *Phagocytosis* of the cornified masses. (5) *Connective-tissue scar*. Two spontaneous mammary carcinomas in mice were treated with X-rays. Several biopsy specimens removed after radiation displayed hypertrophy of the cells, larger acini and numerous cysts which were interpreted as signs of increased secretion. *Clunet* stated that the tumour cells of a human spindle-cell sarcoma produced collagen fibrils after radiation, and that the tumour was transformed into a fibroma. Twenty-two tumours (polymorphous-cell sarcoma) transplanted into mice were treated with 30 H. Transitory degenerative changes were observed in this radio-resistant tumour which, however, failed to show any signs of differentiation after radiation.

Accordingly, *Clunet* maintains that he has observed increased differentiation in epitheliomas, mammary carcinomas, and a spindle-cell sarcoma. However, the essential feature of *Clunet's* observations appears to be the enlargement of the cells. On one of the photographs it is evident that the "marked cornification" is derived from the surface epithelium. The stated differentiation in the spindle-cell sarcoma is no doubt due to confusion with the collagen fibrils of the stroma.

Following X-radiation *Aschoff, Krönig & Gauss* (1913) observed marked cornification in a previously non-cornifying cervical carcinoma and elements resembling squamous epithelium in a tubular mammary carcinoma. These phenomena of metaplasia were interpreted as a change of the cancer cells in the shape of increasing differentiation, their appearance approaching that of normal parenchymatous cells.

Haendly, in 1913, reported increased cornification in several cases of cervical carcinoma following treatment with mesothorium. In another paper from 1921 he emphasized that marked formation of epithelial pearls is only met with in cases which beforehand have a tendency to cornification. A similar increased cornification following radium treatment of cervical carcinoma has been described by *Degrais & Bellot* (1915), *Colwell & Russ* (1915) and *Heyman* (1918).

According to *Alter* (1920) basal-cell carcinoma of the skin and of the uterus remains undifferentiated following radium treatment, unlike squamous-cell carcinoma which shows increased cornification after radiation. Adenocarcinoma is also stated to undergo further differentiation following radiation, forming glands which are hardly discernible from normal glands.

Loeb (1922) found the same changes in skin carcinoma, but in his opinion squamous-cell carcinoma only differentiates following radiation, if the dose has not been sufficiently large to kill the tumour cells.

Loeb does not prove his contention.

Quick & Cutler (1925) have described degenerative cell changes and cornification in 30 instances of metastases from squamous-cell carcinoma in cervical lymph nodes following pre-operative irradiation with X-rays or radium.

In 10 cases of transitional-cell carcinoma they found cytolysis, but no cornification. In the subsequent discussion it was emphasized by *Ewing* that the excessive cornification should be interpreted as a consequence of the radiation.

Biopsy specimens had been removed from the untreated tumour, but only from the primary, not from the secondaries. Therefore the results must be taken with some reservation.

Roussy (1925), *Schwarz* (1925), *Werner* (1925), *Hamperl & Schwarz* (1927), *Askanazy* (1928), and *Jedas* (1928) also report cornification in carcinoma following radiation.

Lahm (1928), studying biopsy specimens from 24 cases of carcinoma of the uterine cervix following radium treatment, repeatedly found increased cornification in ripe and midripe growths which had exhibited some cornification prior to radiation. In the unripe growths the cornification was less marked, but discernible. This increased cornification was sometimes observed after the lapse of 24 hours, but usually it set in simultaneously with visible nuclear changes, reaching its summit when there was excessive shrinkage or break-down of the nuclei. It is described as hyaline or keratoid degeneration, the cells and nuclei being shrunk and the protoplasm granular and highly eosinophilic. Cornification following radiation is stated (1927) to differ from the parakeratosis normally encountered in the neoplastic cells characterized by late affection of the nuclei. It is considered to represent increased function of the tissue constituting a favourable form of spontaneous healing. *Dustin* has reported the same results and found the same explanation to apply following radium treatment of cervical carcinoma (1927). After telecurietherapy of an undifferentiated mammary adenocarcinoma (1929) he observed enlargement of the cells which showed monstrous, large nuclei. This was interpreted as differentiation, although the formation of glandular spaces was not increased. In columnar-cell carcinoma (1930) the ripening following radiation is considered far less pronounced. The explanation given is that the columnar cells may differentiate and dedifferentiate repeatedly and again divide, etc. This should make them more radio-resistant.

Neither *Lahm* nor *Dustin* published any photographs, and it is therefore impossible to control their descriptions. *Lahm's* papers particularly appear to lack sufficient foundation.

Lubarsch & Wätjen in 1928 reviewed the literature published on the subject. They express some doubt as to the contended ripening after radiation, emphasizing that different parts of the same tumour often exhibit a very different degree of maturity. They also find that it is difficult to rule out a secondary effect from cancer cells which are in the process of breaking down and which e. g. may cause metaplasia of glandular epithelium to cornifying squamous epithelium. These workers do not add any studies of their own.

Dyroff (1928) reported degenerative cellular changes following X-radiation of cervical carcinoma, stating, however, in 1929 that when "the dosage is insufficient" one may encounter marked cornification, if the tumour already possesses a tendency to cornification. In this author's opinion the undifferentiated cells disappear in such cases, whereas there is a selective survival

of the differentiated cells which now may show more tendency to cornify, because they only have a slight tendency to proliferate. At this stage the tumour is extremely radio-resistant, but very malignant, as there is a possibility that it may form little differentiated daughter cells equally malignant as the parent cells.

Dyroff's descriptions and conclusions make the impression of being very sober.

Cornification following radiation has also been observed by *Seitz* (1929), *Sculberger, Schmidt & Kröning* (1929), *Schmitz & Hueper* (1930), *Schmitz, Schmitz & Scheehan* (1939).

Gambarow (1931) reports increased cornification in a very slightly cornifying basal-cell carcinoma of the skin following X-radiation.

According to *Regaud* (1931) radiation of cornifying skin carcinoma causes destruction and absorption of the germinative cells, whereas the daughter cells (peripheral to the former) develop into cornified cells, just as is seen after radiation of normal skin. Thus, the cornified masses are increased in order to be absorbed later. *Regaud's* observations appear to be very convincing.

Two days after the radium treatment of a highly differentiated adenocarcinoma in mice, *Ludford* (1932) found enlargement of the cells and granules of secretion. He interpreted both phenomena as signs of higher differentiation. An undifferentiated mammary carcinoma, on the other hand, failed to show signs of secretory activity after radiation.

Sedgwin (1932) found marked cornification in 50 tar cancers in mice following X-radiation. The cornification appeared earliest after large doses, occurring after the onset of degenerative nuclear changes, but prior to complete necrosis of the cells. Only a few biopsy specimens were available from untreated cases.

Frola, in 1933, found cornification in addition to degenerative changes in the cells following radium treatment of carcinoma of the cervix uteri. His series comprised 42 cases. The cornification was most distinct in the *slightly* differentiated tumours. Photographs are not submitted.

Healy & Arneson (1934) removed biopsy specimens twice weekly in 17 cases of cervical carcinoma during a course of fractionated X-radiation (2000—2400 r to each of two fields). One specimen exhibited cornification after the radiations. *Arneson & Stewart* (1935) and *Arneson* (1936) have reported a few similar instances following X-radiation or radium.

The descriptions and photographs appear to indicate degenerative changes in the cells rather than differentiation.

It is discussed by *Stewart & Farrow* (1940) whether such changes and similar changes observed in basal-cell carcinoma of the skin following X-radiation in the form of local foci of "squamous metaplasia" are due to degenerative metaplasia or to "an artificial senescence" in which the cornified cells represent the regular development of the cells. In their material the process was not orderly and regular, not always affecting the oldest, central cells first, but sometimes appearing first in the peripheral zone. Furthermore, the distribution was completely

irregular. Consequently, the phenomenon is not interpreted as an accentuation of the normal senescence.

Transitional-cell carcinoma has been studied by *Schinz & Baumann-Schenker* in 1936 and further by the latter in 1937. In one growth of the tonsil consecutive biopsies removed during fractionated X-radiation showed increasing signs of formations resembling epithelial pearls. It is therefore considered likely that it is a case of genuine cornification.

By histological study of carcinoma of the upper respiratory tract and the oral cavity following fractionated X-radiation, *Friedman* (1939) found an "adaptation" of the tumour cells similar to that reported by *Ewing* (1938). He calls it a "healing process". This may be observed when the dosage is incorrect and may be described as follows: The radiation kills the anaplastic cells, it is true, but the very mature cells remain practically unchanged and an intermediate group of cells are rendered more mature. Consequently there will be not only a relative, but also an absolute increase in the number of mature cells (for instance, there will be a far greater number of epithelial pearls in a Grade III carcinoma of the tongue after the radiation). This "healing process" is stated to take place during a healing phase which begins between the 21st and 28th day in undifferentiated tumours of the upper respiratory tract (rather later in differentiated tumours) and lasts for 4–6 weeks. According to *Friedman* a strong healing process may make the tumour so mature (and consequently also radio-resistant) that it responds like the normal skin and may regenerate in spite of continued radiation. The healing phase should therefore be evaded, and he recommends radiation before its onset or after its termination. The radiation of undifferentiated tumours should be completed in the course of 3–4 weeks, whereas differentiated tumours should be radiated for 4 weeks. Renewed radiation should then follow after an interval of 4 weeks. As a daily dose he suggests about 200 r, preferably not less than 100 r, whereas he considers the quality of the rays and protraction to be of minor importance in this connection.

In another paper from 1940 *Friedman* emphasizes that it is not only possible to observe maturation of tumour cells in the tardy healing phase of a residual tumour, but that apart from causing alterations in the mitosis, X-rays and radium induce rapid maturation in the form of parakeratinization or cornification of epithelial tumour cells. Morphologically, this early maturation of the cells (which may occur in a few days) resembles the late maturation in the healing phase. Apart from studying tumours of the upper respiratory tract, he also investigated the maturation of the cells in cervical carcinoma.

Friedman's material is not reported in details, but it seems that at any rate part of his conclusions are based on the observation of the reaction of the normal oral mucosa to radiation.

It is stated by *Ellinger* (1941) that cornifying squamous-cell carcinoma is only partly destroyed by cytolysis. The remaining cells ripen and differentiate into keratin cells. Histological changes in sarcoma following radiation are seldom observed and are not characteristic. They are said to resemble spontaneous changes in the growths.

This author does not report any experiments of his own.

According to *Warren* (1941 and 1942, 1) the increased radio-resistance so often observed in a recurrence following radiation may be caused partly by an alteration in the stroma and in the vascularization of the tumour, partly by the general condition of the patient, and partly by a selective survival

of radio-resistant cells, whereas the radio-sensitive ones are killed. In an excellent survey of radiation changes in malignant tumours from 1944 he states that the cytoplasm of squamous-cell carcinomas has a tendency to cornify during radiation, the kerato-hyaline granules and horny cell membranes being increased. In addition there will be more intercellular bridges. He does not state whether the increased cornification is interpretable as a sign of increased differentiation.

In a paper from 1941 *Glücksmann* has reported studies of the histological reactions observed in the tumour cells following radiation with special reference to differentiation after radiation.

This author has used a quantitative histological analysis worked out by himself. Only the youngest cells were selected and examined, the author counting and classifying the cells in selected, narrow projecting areas, whence growth takes place. The cells were classified into four groups:

- (1) *Mitotic cells* (including all mitoses, abnormal as well as normal).
- (2) *Resting cells*.
- (3) *Differentiating cells*:
 - (a) *Cells in the process of keratinization* (seen when the cytoplasmic structure is changing and when the preparation is stained blue with Heidenhain's azan).
 - (b) *Cells increasing in size*.
- (4) *Degenerate cells*:
 - (a) *Cells showing primary nuclear disintegration*. (Chromatopyknosis, hyperchromatosis, or chromatolysis). Stated to be usually derived from abnormal mitoses.
 - (b) *Cells showing nuclear degeneration secondary to degenerative cytoplasmic changes*. Usually originating from resting and differentiating cells, the number varying with the degree of differentiation.

It is stated that these four categories are present in every tumour in characteristic proportions. *Glücksmann* studied human tumours from which he removed 2—5 biopsies during or after therapeutic radiation. Afterwards, these biopsies were dealt with as a whole, tumours of the same histological type and receiving the same dose being grouped together. From each tumour 500 cells from selected "young" areas were counted. The examination of the radiated tumours revealed the following:

(1) *Mitotic cells*: There will always be a reduction in mitosis, the percentage of mitotic cells often reaching 0 in the course of rather more than an hour. Later there will be an increase in spite of repeated radiation, and now there are many abnormal mitoses. Many of these abnormal cells break down and supplement the degenerate cell count. Later still, the mitotic figures disappear altogether.

(2) *Resting cells*: Their number is nearly always reduced. They may differentiate or break down in mitosis in order later to increase the number of degenerate cells.

(3) *Degenerate cells*: Increase in number following radiation, usually after mitosis has reached its minimum. In basal-cell carcinoma the rise may be very rapid, particularly in the absence of any attempt at differentiation, all the affected cells tending to break down when division is attempted.

(4) *Differentiating cells*: In most cases of squamous-cell carcinoma *Glücksmann* reports an increase in the number of differentiating cells, corresponding to the decrease in the number of resting cells (so long as the differentiating cells do not degenerate). His series included 7 cases showing increasing differentiation following radiation (2 radium-treated basal-cell

carcinomas, 3 squamous-cell carcinomas of which 2 had received X-rays and 1 radium, and 2 cases of carcinoma of the cervix uteri treated with combined radium and X-rays). The increase in differentiation was most marked in cases which already showed pronounced differentiation (resting cells = differentiating cells). The nature and degree of differentiation is stated to vary within wide limits. Cells which are enlarged or maybe monstrous following radiation are grouped with differentiating cells, as they are not met with according to *Glücksmann*, where the radiation does not alter the proportion between resting and differentiating cells, like for instance in basal-cell carcinoma.

Glücksmann states that the object of the therapy is *inter alia* to increase the number of differentiating cells at the cost of the resting cells.

Broders' grading is considered valuable in evaluating untreated tumours, whereas horny masses, parakeratosis or epithelial pearls in radiated squamous-cell carcinomas are not taken to express the degree of differentiation adequately and are not dealt with. In *Glücksmann's* opinion it has not yet been definitely elucidated what makes a radiated cell attempt mitosis and perish or differentiate. It is emphasized that a malignant growth should be judged not only in terms of its pathological classification including *Broders'* grading, but also in terms of the number of cells in each of *Glücksmann's* 4 categories. It is stated that the effectiveness of the radiotherapy can be judged on the basis of the latter.

The essential observations made in *Glücksmann's* material appear to be those of an increase in the size of the cells (taken to be a form of differentiation), whereas cornification was only increased to a slight extent. Epithelial pearls or parakeratosis are not mentioned at all. Among other possible sources of error, one is struck by the fact that the central part of the tumour is not counted at all. *Glücksmann* only counted the youngest areas, in other words he used an elective counting, and it cannot be ruled out that some of the radiated cells have escaped counting.

Glücksmann & Spear, in 1945, employed the same quantitative histological method in the examination of a series of 166 patients with carcinoma of the uterine cervix.

They removed biopsies before and during treatment with X-ray and radium (by the Stockholm technique). According to these workers a favourable response to the radiation, usually leading to healing, without recurrence, is characterized by early disappearance of mitotic and a little later of resting cells with an increase in the number of differentiating and degenerate cells. Later still, there will be a decrease in the differentiating cells, when the fully differentiated cells die and become absorbed. Correspondingly, there will be an increase in the degenerate cell count.

The tumours are divided into (1) anaplastic parakeratotic tumours, (2) anaplastic squamous-cell tumours, and (3) anaplastic columnar-cell tumours. The histological response of the 1st group to radiation is usually poor (there is *inter alia* seldom differentiation) and the 5-year results are less satisfactory than in the other 2 groups. In the 2nd group increased differentiation and degeneration following radiation is far more common, whereas the 3rd group exhibits "mucinous differentiation" and a response similar to the 2nd group. In the two latter groups the 5-year results are fairly satisfactory.

It is emphasized that one must be careful not to draw conclusions on the

basis of the radio-sensitivity of normal immature tissue and make them apply to malignant anaplastic tumours, as the growth of the normal cell, unlike that of the tumour cell, is terminated by differentiation. Therefore, the cells of a malignant anaplastic tumour are not easily cured by radiation, although there may be an early temporary response. The more highly differentiated tumours, on the other hand, may differentiate further and therefore the prospects of cure are more favourable. Moreover, they contain fewer cells which are able to divide.

The same objections can be raised to this work as to *Glückmann's* earlier paper. The technique is very refined, but the numerical differences are small and not very convincing. In addition, sources of error may arise because of the elective counting and the omission of parakeratosis, cornification, and epithelial pearls. The increased differentiation of the adenocarcinomas is based on increased production of mucin, whereas nothing is stated about increased glandular imitation.

These studies have been continued by *Koller & Smithers* (1946) who also maintain that they have observed increased differentiation in several cases examined by the quantitative histological method. In the opinion of *Koller & Smithers* it has not been proved so far that differentiation is a direct effect of radiation, and that possibly it is merely relative as the result of tumour breakdown. It is, however, emphasized by *Smithers* (1946) that presumably radiation alters the metabolic processes in the cell which consequently differentiates and loses its capacity to proliferate. Differentiation following radiation has also been observed in basal-cell carcinomas.

Koller & Smithers' own investigations are not substantiated.

Donaldson (1946), like *Glücksmann*, considers large flat cells with an eosinophilic protoplasm to be differentiated and regards increased differentiation following radiation as a good prognostic omen. He does not report any studies of his own.

(3) Discussion.

It will be seen from the literature reviewed above that the histological investigations made so far have given conflicting results, one group of workers supporting the opinion of *Perthes* and *Apolant* (1903 and 1904) that the histologically demonstrable radiation reactions in cancer cells indicate degeneration, and another group supporting the theory advanced by *Dominici & Barcat* (1907) that the main effect of X-rays and radium on cancer cells is due to a radiation-induced increased differentiation (ripening) of the tumour cells. As a fully differentiated cell cannot divide and thereby give rise to new neoplastic growth, it must, according to the theory, degenerate and die, partly because of a degenerative injury caused by the radiation. Accordingly, the advocates of the theory of differentiation do not rule out the presence of degeneration of the cancer cells, but they attach most importance to the contended differentiation.

The best founded works supporting the theory of degeneration are those of *Melnick & Bachem* (1937), *Englmann* (1938), and *Lacassagne & Gricouroff* (1941). The theory of differentiation is not directly mentioned by any of these workers, however, and their studies do not appear to have been made for the purpose of investigating this particular phenomenon among histologically demonstrable radiation injuries.

At the beginning of this century the theory of differentiation was studied mainly by French workers, *Dominici & Barcat* (1907, 1909) and *Clunet* (1910), later by *Lahm* (1927, 1928) and *Dustin* (1927—1930). Recently it has received support from American workers, *Friedman* (1939, 1940) and *Warren* (1944) and in major English works (1941—1946) by *Glücksmann*, *Spear*, *Koller*, *Smithers* and *Donaldson*.

Doubts as to the correctness of the theory of differentiation have, however, been advanced *inter alia* by *Lubarsch & Wätjen* (1928), *Stewart & Farrow* (1940) and in 1946 by *Koller & Smithers*. These critics emphasize, among other things, that the increased differentiation observed may be merely relative as a result of tumour breakdown.

The vast majority of the workers base the theory of differentiation on cornification in squamous-cell carcinoma. While *Frola* (1933) found that increased cornification was in most cases encountered in tumours with low grade differentiation, a number of others (*Alter* (1920), *Haendly* (1921), *Lahm* (1928), *Dyroff* (1929), *Friedman* (1939), *Glücksmann* (1941) and *Glücksmann & Spear* (1945)) maintain that cornification following radiation is most marked, when the tumour already possesses a certain tendency to cornification. The influence of the dosage has been studied by *Loeb* (1922) and by *Dyroff* (1929) both of whom state that differentiation only occurs after radiation, if the dose has not been sufficiently large to kill the tumour cells. According to *Friedman* (1939) differentiation may result from a kind of "adaptation" of the tumour cells, provided that the tumour is radiated with too small daily doses (below 100 r) or too long (longer than 3—4 weeks), and he also states that differentiation may be observed if the treatment has to be stopped. In the literature available it has not been possible to find any indication of a fundamental difference between the capacity of X-rays and radium to produce differentiation.

(4) Planning of My Investigations:

None of the earlier papers, which usually have been based on observations of individual cases or minor series, have afforded a satisfactory answer to the question as to whether or not differentiation

takes place during radiation with X-rays or radium. The problem must be said to be of significance, not only in theory, but also in practical radiotherapy, as it has been emphasized by several authors (*Glücks-mann* (1941), *Glücks-mann & Spear* (1945) and *Donaldson* (1946)) that the increase in differentiation, which perhaps is obtained, is a valuable prognostic sign which should be observed closely during treatment, whereas *Friedman* (1939—1940) regards differentiation following radiation to be harmful and recommends a special technique to avoid it.

I therefore set myself the task of studying differentiation in cancer cells following therapeutic X-rays and radium on a broad basis. For this end a number of characteristic types of tumours which theoretically should have a possibility of exhibiting histologically demonstrable differentiation were examined before, during, and after radiation. It having been established by all workers that the effect of X-rays and radium is fundamentally the same, the histological reactions observed in the human material following X-radiation and radium will be considered together. For practical reasons the animal experiments were only carried out with X-radiation.

The human material comprises biopsies and operation specimens from cancer patients treated with X-rays or radium at the Radium Centre in Copenhagen according to the customary principles of that Clinic.

The animal material consists of tumours transplanted into mice and radiated with a technique as close as possible to the fractionated X-radiation used in human cancer. In addition a number of treatments were carried out in one sitting, with a dose ranging from 25 to 2000 r.

Differentiation is easily recognizable in squamous-cell carcinoma in the form of the various phases of cornification, and the starting point of this work was a transplantable squamous cell mouse carcinoma. In the first passages it had shown marked cornification in order later to change its character in the direction of an undifferentiated squamous-cell carcinoma, showing merely by sporadic occurrence of parakeratosis or cornification that the tumour cells had preserved a certain capacity for differentiation. Theoretically, this tumour is close to the ideal for experiments of this kind, offering the best conceivable possibilities of revealing a histologically demonstrable differentiation. Therefore, a number of X-radiation experiments were made with this tumour.

In addition, histological examinations were made of human skin carcinoma and carcinoma of the cervix uteri following treatment with X-rays and radium.

Adenocarcinoma was studied with a view to increased glandular formation, partly mammary carcinomas from the patients of the Radium Centre, and partly an undifferentiated transplantable mam-

mary mouse carcinoma which in a few cases had shown a capacity for further differentiation.

As to sarcoma, I chose an undifferentiated spindle-cell sarcoma transplantable into mice, examined for a tendency to form collagen fibrils following X-radiation, and a transplantable chondrosarcoma in which differentiation, if any, should be demonstrable in the shape of increased formation of cartilaginous intercellular substance, perhaps also in the shape of bone formation.

It was not considered justifiable to study human sarcoma following radiotherapy, as this would involve the removal of serial biopsies during treatment.

Tumour cells derived from the bone marrow were not studied, as it is difficult to demonstrate differentiation, if any, in these tumours. On the whole, bone marrow is not suitable for investigations of this nature, as it is impossible to be certain of a uniform distribution of the dose throughout the tumour or to rule out toxic actions or admixture of blood. The same objections apply to the use of normal bone marrow in which *Denstad* (1943) maintains to have observed differentiation following treatment with X-rays and radium. As it is, in addition, a question of normal blood cells and their preliminary stages and not of cancer cells, such studies fall without the scope of this paper.

MY INVESTIGATIONS

CHAPTER III

Technique.

(1) Tumours Transplanted into Mice.

(a) *Animal material:* The mice used were of strains Aka Furth, Dlb (a subline of *Little's dba*), and Street. The first two strains are inbred, the third only partly inbred. More detailed information about these strains of mice can be found in *Stamer's* thesis from 1943. The mice were kept in zinc cages, 3—5 of the same sex in each, temperature about 20° C. They were fed rolled oats, whole wheat and milk to which was added a mixture of lucerne meal, casein, dry milk, cod liver oil and salt. The mice were weighed 2—3 times weekly.

(b) *The tumour material* will be described under the individual headings.

(c) *Technique of transplantation:* The mice from which the tumours were obtained were killed, either with gas or by stretching the neck and the whole mouse immersed in alcohol, 70 per cent., for $\frac{1}{4}$ minute. The tumour was removed with sterile instruments, cut until it had the consistency of thick juice, and without being diluted it was drawn into a sterile screw syringe and injected through a coarse needle subcutaneously into the axilla (about 0.05 cc.), intramuscularly into the left thigh (about 0.04 cc.), or subcutaneously into the dorsal aspect of the tail (about 0.01 cc.) 1—2 cm. from the root of the tail.

(d) *Measurements of the tumours.* The takes were measured with a slide gauge in 3 dimensions. In the irradiation experiments the radiated tumours as well as the controls were measured daily. As far as the axillary and femoral tumours are concerned, the measurements were rather rough, whereas they were considerably more accurate in the case of the growths on the tail. However, all the measurements allow of an estimate of the susceptibility of the tumour to irradiation.

The cages were inspected daily and all dead or killed mice autopsied. All tumours and metastatic deposits, if any, were removed *in toto* and fixed for histological examination.

All the transplantations, measurements and autopsies were made

by my wife or myself. Numerous control measurements showed that there was no difference between the measuring technique used by my wife and by myself.

(e) *Preparation of removed tumour tissue:* The great majority of the tumours were fixed in formalin-alcohol, 1—3, for 12—24 hours, but a few were fixed in formalin-saline solution, 10 per cent. The specimens were embedded in paraffin and stained partly with hematoxylin-eosin and partly by the method of *van Gieson-Hansen*.

In some instances keratin staining was employed, partly *Gram*-staining and partly *Heidenhain's* azan staining. Often several sections were taken in various planes throughout the tumor.

(2) Human Tumours.

The histological examinations were performed on biopsies (excision biopsies or punch biopsies) removed at varying intervals after the radiation and on operation specimens from patients treated at the Radium Centre. Nearly all the biopsies were removed by me, as far as possible from the same part of the tumour, and, whenever possible, from the edge of the tumour. The specimens from the mammary growths were, however, removed by Dr. *S. Kaas*.

All the preparations were fixed in formalin-alcohol, embedded in paraffin and stained with hematoxylin-eosin and according to the method of *van Gieson-Hansen*.

(3) Radiation Technique.

(a) *Animal material.* The tumours were radiated locally at the Radium Centre with its Apparatus 8, a Siemens Bomb, maximum voltage 193 kv., 20 ma. $\frac{1}{2}$ mm. copper filter, half value layer 0.85 mm. Cu., focal-skin distance 50 cm., intensity 32 r/min., no applicator employed. The only exception is Series 163 a (squamous cell carcinoma in Aka mice) in which 3 of the treatments were given with Apparatus 11 or 12, both with a kilovoltage of 180, 6 ma. $\frac{1}{2}$ mm. Cu., half value layer 0.9 mm. Cu., intensity 18.8 and 14.7 r/min. respectively.

During the radiation the mice were stretched out on a wooden board, 3 cm. thick, on a high wooden foot-stool below the tube. The mice were protected with lead sheets, 3 mm. thick, leaving only the tumour and its immediate surroundings uncovered (tail and thigh respectively in case of the tumours transplanted into the tail or thigh). All the tumours were placed at a distance of about 10 cm. from the middle of the board.

The dose was 32 r/min. measured in air by the method of *Küstner*. Reflection of the rays from the board results in an increase in the dose, found to amount to about 25 per cent. when measured with a

Siemens thimble dosimeter placed in the direction of the central ray. Towards the periphery of the board there was a decrease in the dose, found to amount to about 15 per cent. at a distance of 10 cm. when measured with Siemens dosimeter. By means of small condimeters I found a decrease in the dose of about 20 per cent. under similar radiation conditions, that is approximately the same as found by *Zuppinger* (1928) and *Thayssen* (1945).

Accordingly, under these experimental conditions the dose delivered to the surface of the tumours was equally high or up to about 10 per cent. higher than the 32 r/min. found by the method of *Küstner*.

The tumour dose was not measured, but it was estimated to be very close to the dose delivered to the surface measured in air, as it was a question of very small tumours (with a transverse diameter seldom exceeding 10 mm.) only covered with thin, partly hairy skin, and as the rays used were very hard. By direct measurements with Sievert chambers placed in the abdominal cavity of mice under similar conditions, *Refslund Poulsen* (1944) found the tumour dose to be slightly higher due to the scattering (though not exceeding 20 per cent.) than the dose measured in air. The tumour dose in the axillary tumours was found to be practically equal to that measured in air. *Denstad*, in 1946, found that at a depth of 1 cm. the tumour dose used in ordinary therapeutic radiation ($\frac{1}{2}$ mm. of copper, 170 kv., focal-skin distance 50 cm. and a field of 400 square cm.) was 10 per cent. higher than the surface dose because of secondary radiation.

(b) *Human material*. The radiation technique, the customary method of the Radium Centre, will be described under the individual headings.

CHAPTER IV

Squamous-Cell Carcinoma Aka (Transplantation Experiments with Mice).

(1) Material:

These experiments were carried out with a squamous-cell carcinoma transplanted into Aka mice. Originally, this tumour arose in an Aka mouse which previously had been painted on the back with 9,10-dimethyl-1,2-benzanthracene. Seven months later a growing nodule was felt in the left cheek without demonstrable connection with the skin. Presumably the tumour had arisen in the oral mucosa or perhaps in the excretory duct of a gland. It was transplanted into other Aka mice in which it took in about 100 per cent. of the cases. In 1943 I succeeded in transplanting it into Street mice, but the percentage of takes was far lower. This tumour was unfortunately eradicated by the mouse epidemics which raged in Copenhagen in 1944, before the experiments were entirely concluded.

This rare type of tumour was described by *Engelbreth-Holm* in 1944 as "a transplantable cornifying squamous-cell carcinoma in mice". Histological examination of the original spontaneous growth (Fig. 1) showed a typical cornifying squamous-cell carcinoma (*Broders'* Grade 1) with marked cornification throughout (including numerous keratohyaline granules and large central horny pearls in all the islets of tumour cells). In addition there was a trace of parakeratosis. In the 1st and 2nd passages the cornification remained very pronounced (see Fig. 2), though somewhat less so than in the spontaneous tumour. In the following passages the nature of the tumour changed rapidly, and already in the 3rd—4th passages the tumour was predominantly an undifferentiated squamous-cell carcinoma (see Fig. 3) which, however, contained a certain number of horny pearls (+ keratohyaline granules) and a trace of parakeratosis. In the subsequent approximately 10 passages the differentiation decreased further, and since then it has mostly been an undifferentiated growth, showing, however, through all the passages (it was eradicated in the 91st passage) traces of parakeratosis and cornification in the form of scattered parakeratosis of a few individual cells or a few parakeratotic or horny pearls. The tumour is made up of islets or strands

of medium-sized cells of epithelial appearance with round, hyperchromatic, moderately polymorphous nuclei containing numerous normal or slightly abnormal mitotic figures. The cytoplasm is rather sparse, pale and ill-defined. At an estimate the tumour can be allocated to *Broders'* Grade 3. It does not resemble Krompecher's basal-cell carcinoma, but the slightly differentiated squamous-cell carcinomas seen for instance in the oral cavity and oesophagus in man.

As the differentiation was reduced, the tumour acquired a more marked tendency to necroses and cystic breakdown, and its malignancy increased, the latent period being shortened and the survival time of Aka mice implanted with axillary tumours being reduced from 3 months to 1 month. Another sign of increased malignancy was the fact that now the tumour could be transplanted into another strain (Street), although the percentage of takes was low.

It is well-known that transplanted tumours change their nature during the passages, acquiring a higher malignancy (higher percentage of takes, shorter latent period, more rapid growth, and a consequently shorter survival time of the host). According to *Engelbreth-Holm* the most probable explanation of this phenomenon is an inhibition of the rate of differentiation and perhaps also of other cellular functions during the passages through the various foreign organisms. This appears to be the most adequate explanation in this case. There is no reason to presume that the original tumour has been made up of a mixture of highly malignant (= undifferentiated) and less malignant (= more differentiated) cells and that the most malignant (undifferentiated) cells have become dominant by "selection". In the case under discussion it is not either a question of a mixture of more or less malignant cells in the original growth, but of an almost organoid mass of epithelial cells, differentiating, uniformly and side by side, into flat, cornified cells. The sporadic differentiation encountered in the later passages does not either bear the marks of a mixture, but in an otherwise undifferentiated islet of tumour cells individual cells may differentiate and attempt a more or less successful imitation of the horny pearls in the original tumour.

Theoretically, this tumour should be close to the ideal experimental object to elucidate whether or not malignant tumours are capable of differentiating during radiation. Originally the tumour was highly differentiated, but during continued transplantation its differentiation was considerably reduced and arrived at a fairly constant low differentiation. A preserved capacity for further differentiation may, however, be seen from the frequent finding of traces of differentiation in an otherwise undifferentiated tumour and from the even marked differentiation occurring in places in some of the controls (see Fig. 4). Moreover, the characteristics of differentiation in the type of tumour are fairly easy to recognize in ordinary histological examination, and controls from the same transplantation series afford a basis

for comparison. The fact that we are dealing with a mouse tumour should not be of major consequence, as there is nothing to support a presumption that the differentiation of tumour cells should be governed by other laws in the mouse than in man. It is, however, a disadvantage that the experiments were not performed with spontaneous tumours, but in experiments of this particular nature, this disadvantage is in my opinion by far outweighed by the advantage afforded by a large material derived from the same tumour.

(2) Investigations into Alterations in Differentiation

Following X-radiation:

(a) *Histological appearance of the tumour:* The squamous-cell carcinoma was transplanted by me at the Radium Centre from the 54th passage until it was eradicated in the 91st passage. As Aka mice were not available in sufficient numbers, the experiments during the first 9 months were carried out with Street mice which had a very low percentage of takes (about 20—25 per cent.). Using 1000 Street mice a total of 47 tumours were radiated in 7 experiments. Because of the small number of tumours and their inconstant growth, the experiments are not homogeneous, and their value is further reduced by the insufficient number of controls. Therefore, all the experiments made with Street mice have been left out of this work. The conclusions which — with certain reservations — could be derived from the experiments with Street mice do not differ essentially from those based on the later experiments with Aka mice and which will be reported below. The several hundreds of “controls” afforded by the Street mice by microscopic examination of all takes did not either show any divergences from the histological appearance in the Aka controls. In other words, the differentiation of the tumour did not change its character during the two years while I was studying it.

The tumours were transplanted partly subcutaneously into the axilla, partly into the femoral muscles, and partly subcutaneously into the tail. Unlike the tumours in the first two sites, the growths on the tail only took in Aka mice and they appeared to grow more slowly and after a longer latent period. Histologically they often exhibited a somewhat divergent appearance, containing more pyknotic nuclei and more small, angular nuclei in the tumour cells; in addition, they usually exhibited fewer mitoses and a more ample connective-tissue stroma. These changes were presumably due to the poorer conditions of growth. It is my impression that the tumours on the tail showed rather more tendency to differentiate than those of the axilla and thigh, but I am unable to submit a definite proof. The few pulmonary metastases which occurred were undifferentiated.

It does not appear to have influenced the differentiation of the tumours whether the hosts were Street or Aka mice.

The tumours were fixed in the usual way and studied after the two standard stainings with hematoxylin-eosin and *van Gieson-Hansen*. In a certain number of cases the preparations were, in addition, stained with *Gram's* stain: Carbol gentian violet, iodine, hydrochlorid acid-alcohol, and carbol fuchsin. This is, however, inferior to the two first-mentioned staining methods as regards morphological details, and only keratin stains blue regularly, whereas parakeratosis is usually (but not always) decoloured by the subsequent differentiation in hydrochloric acid-alcohol. In a few cases I also used *Heidenhain's* azan (see *Romeis* 1932) which stains the cytoplasm of the tumour cells a greyish blue, parakeratosis and keratin (cornification) a vivid red. This method did not either in my investigations yield results which were comparable as regards accuracy with the two standard methods which afford a good staining of parakeratosis and keratin.

A number of tumours were studied in several sections at different depths throughout the tumour.

The grade of differentiation was estimated on the basis of the proportion between the amount of parakeratosis and keratin and the amount of undifferentiated tumour tissue. The size of the cells or polymorphism, on the other hand, were not included in the estimate of the grade of differentiation.

The first sign of beginning differentiation is *parakeratosis*: The cytoplasm becomes more abundant, eosinophilic (staining a bright yellow with *van Gieson-Hansen*) and at the same time the previously homogeneous cytoplasm will become condensed. The nuclei are at times slightly enlarged, but soon become pyknotic and more narrow and at the same time large lumps or lamellae of an eosinophilic substance are formed in the cytoplasm. In some instances there will be concentric parakeratotic pearls around one or more of these highly changed epithelial cells. The formation of such pearls does not appear to give rise to further differentiation above what is seen in a parakeratotic plaque. Often the difference depends merely on the direction of the section.

In some cases actual *cornification* (= keratinization) is seen in the tumour: The cells grow larger and flatter, and perhaps keratohyaline granules are formed. Later the cytoplasm becomes abundant, eosinophilic, containing horny (keratinized) lamellae or lumps, while the nucleus becomes quite narrow in order later to disappear entirely. Frequently one may see concentric horny pearls of an appearance exactly like the parakeratotic ones with the exception that the nuclei have disappeared. As cornification I have included the (common) cases in which keratohyaline granules are not demonstrable before the cornification (unlike for instance *Kyrle* (1925) and *Bang* (1941)), as there appears to be an even transition from cornification without

or with only a few keratohyaline granules to a fully developed stratum granulosum. Similarly, one may say that the tumour shows an at any rate fairly even transition between parakeratosis and cornification, the essential morphological difference being the presence or absence of the nuclei. However, it seems to me to be practical and in most cases possible to make a distinction between parakeratosis and cornification. Actual cornification must be considered a sign of higher differentiation in the tumour than parakeratosis in which the epithelial pearls are formed before the nucleus is destroyed. In this connection it must be pointed out that parakeratosis does not occur in the course of the normal cornification of the skin, but in a number of diseases such as psoriasis, and it is interpretable as *abnormal differentiation*.

The examinations have included the entire tumour, also the partly necrotic areas, if only the cellular details have been discernible. The most common finding was squamous-cell carcinoma without any trace of parakeratosis or cornification. Next in order of frequency was squamous-cell carcinoma with a trace of parakeratosis or (and) cornification in the form of single or quite few plaques with slight parakeratosis, cornification or 1—2—3 small epithelial (parakeratotic or horny) pearls. It appears to be a matter of chance whether a section reveals no tendency to differentiation or traces of differentiation, as numerous examinations of new sections from the same tumour have revealed now traces of differentiation, now none. In a few cases there will be a more marked parakeratosis and cornification in places with numerous epithelial pearls (parakeratotic and horny) and abundant parakeratosis and keratin. In such cases new sections were examined from deeper down in the block; they showed no or only traces of parakeratosis or cornification. These instances of a more pronounced differentiation were all found in tail tumours. Intercellular bridges were never observed in this tumour at all.

In microscopic examination of the radiated tumours it is usually easy to discern parakeratosis and cornification and to assess the amount, as hematoxylin-eosin as well as *van Gieson-Hansen* both afford a good staining of horny tissue. The difficulty in the differential diagnosis from hyalinized connective tissue may with some experience be eliminated by means of the purely morphological structure in a differentiated squamous-cell carcinoma. The pearls, parakeratotic as well as horny and the regular cornification are unmistakable, whereas parakeratosis without pearls may in certain cases be difficult to discern definitely from cellular degeneration in the radiated tumours. Following radiation the entire cell is enlarged, nucleus as well as cytoplasm, and the cytoplasm acquires a tendency to become eosinophilic. As long as the cytoplasm remains homogeneous I do not interpret this as a sign of beginning cornification or parakeratosis, but merely as a kind of hydropic state in the cytoplasm and a link in the cellular

degeneration following radiation. The assumption that this is not a question of incipient cornification is supported by the fact that it may be observed in tumours where cornification is out of the question (for instance in the solid mammary carcinoma in Dlb mice). In exceptional cases it was, however, impossible to judge the differentiation of the cytoplasm with certainty, and such cases are listed as doubtful parakeratosis. It has been a question of very small and sparse changes in a few cells. No help has been afforded by *Gram* or *Heidenhain* staining, partly because the changes were so vague that they were not discernible in new sections. Another difficulty was the increasing necrosis in the radiated tumours for which reason certain parts of the tumour could not be assessed with certainty. In these cases the purely morphological structure also proved to be applicable in deciding the issue.

(b) *Radiation technique.* The radiations were carried out with the technique described in Chapter III.

A total of 108 mice were radiated, whereas 84 served as controls.

(c) *Controls:* The 84 controls represent untreated tumours from the period covered by the radiation experiments (from the 71st to the 91st passage):

- | | |
|--|----|
| (1) Actual control tumours (from the same series as the mice treated with X-rays) (Nos. 38—51, 53—61, 63—74, 76—79, 81—84, all in Aka mice; 36 tumours of the tail, 4 of the thigh, and 3 of the axilla) | 43 |
| (2) Tumours used for transplantation into the radiated series (Nos. 37, 52, 62, 75, 80, 3 Street and 2 Aka mice; 1 tumour of the thigh, 4 of the axilla) | 5 |
| (3) Tumours from the successive transplantation series (Nos. 1—36, 12 Street and the remainder Aka mice; all the tumours had their site in the axilla) | 36 |

The first two groups of controls will be described together with the tumours radiated within the same series.

Upon histological examination of the controls from the successive transplantation series (all tumours of the axilla) from the period covered by the radiation experiments, 30 were found to be entirely undifferentiated without any signs of parakeratosis or cornification, whereas 5 showed traces of parakeratosis or cornification and 1 both. In most instances the tumour was, however, used for transplantation and therefore microscopic examination could only be made of part of it. Accordingly, one cannot expect to find signs of cornification in these cases as frequently as in the actual controls. The same applies to those transplantation tumours which were used for transplantation into the radiated series. In addition, it is a question of axillary tumours which appear to exhibit rather less differentiation than tumours of the tail.

(d) *Radiation experiments:* All the experiments were carried out

with Aka mice, series 139 a exclusively with femoral tumours, the remaining series with tumours of the tail.

Radiation in Single Doses:

Single dose of 100 r (Series 176 a).

Controls: A total of 15 (Nos. 37—51). Twelve squamous-cell carcinomas without parakeratosis or cornification, 1 squamous-cell carcinoma with doubtful parakeratosis (No. 43) and 2 squamous-cell carcinomas with a trace of parakeratosis (Nos. 38, 39).

Radiated tumours: A total of 9 (Nos. 85—93). Seven squamous-cell carcinomas without parakeratosis or cornification, 2 squamous-cell carcinomas with a trace of parakeratosis (Nos. 87 and 91).

Single dose of 300 r (Series 163 c).

Controls: A total of 4 (Nos. 52—55). One squamous-cell carcinoma without parakeratosis or cornification (No. 52), 1 with a trace of parakeratosis (No. 54), and 2 with a trace of cornification (Nos. 53 and 55).

Radiated tumours: A total of 5 (Nos. 94—98). All squamous-cell carcinomas without parakeratosis or cornification.

Single dose of 400 r (Series 173 f).

Controls: A total of 6 (Nos. 56—61). Four squamous-cell carcinomas without parakeratosis or cornification, 2 squamous-cell carcinomas with a trace of parakeratosis and cornification (Nos. 56 and 57).

Radiated tumours: A total of 8 (Nos. 99—106). Four squamous-cell carcinomas without parakeratosis or cornification, 2 squamous-cell carcinomas with a trace of parakeratosis (Nos. 103 and 105), 1 squamous-cell carcinoma with a trace of cornification (No. 100), and 1 squamous-cell carcinoma with moderate parakeratosis and slight cornification in places (No. 101). New sections from deeper down in the block: Squamous-cell carcinoma without parakeratosis or cornification.

Single doses of 500, 1000, 1500 and 2000 r (Series 159 a, b, c, and d).

Controls: A total of 13 (Nos. 62—74). Seven squamous-cell carcinomas without parakeratosis or cornification, 3 with a trace of parakeratosis (Nos. 65, 68, and 74), 1 with a trace of cornification (No. 67), and 2 with a trace of parakeratosis and in places slight cornification (Nos. 72 and 73).

Radiated tumours:

Single dose of 500 r: A total of 4 (Nos. 107—110). All squamous-cell carcinomas without parakeratosis or cornification.

Single dose of 1000 r: A total of 5 (Nos. 111—115). One squamous-cell carcinoma without parakeratosis or cornification (No. 112), 1 with a trace of parakeratosis (No. 113), 2 with a trace of parakeratosis and cornification (Nos. 111 and 115) and 1 with slight parakeratosis and cornification in places (No. 114).

Single dose of 1500 r: A total of 4 (Nos. 116—119). Two squamous-cell carcinomas without parakeratosis or cornification (Nos. 116 and 119), 1 with doubtful parakeratosis (No. 118) and 1 with a trace of cornification (No. 117).

Single dose of 2000 r: A total of 5 (Nos. 120—124). Two squamous-cell carcinomas with a trace of parakeratosis (Nos. 120 and 121), 1 with a trace of parakeratosis and cornification (No. 122), 1 with slight parakeratosis in places (No. 124), and 1 with slight parakeratosis and cornification in places (No. 123, see Fig. 5).

Single doses of 500, 1000, 1500, and 2000 r (Series 173 b, c, d, and e).

Controls: The same as those used in the experiment with a single dose of 400 r.

Radiated tumours:

Single dose of 500 r: A total of 4 (Nos. 125—128). Two squamous-cell carcinomas without parakeratosis or cornification (Nos. 127 and 128), 2 with a trace of parakeratosis (Nos. 125 and 126).

Single dose of 1000 r: A total of 4 (Nos. 129—132). One squamous-cell carcinoma without parakeratosis or cornification (No. 129), 2 with a trace of cornification (Nos. 131 and 132) and 1 with a trace of parakeratosis and cornification (No. 130).

Single dose of 1500 r: A total of 4 (Nos. 133—136). One squamous-cell carcinoma without parakeratosis or cornification (No. 133), 2 with a trace of parakeratosis (Nos. 135 and 136) and 1 with moderate parakeratosis and cornification in places (No. 134); new sections from deeper down in the block: No parakeratosis or cornification.

Single dose of 2000 r: A total of 4 (Nos. 137—140). One squamous-cell carcinoma without parakeratosis or cornification (No. 140), 2 with a trace of parakeratosis (Nos. 137 and 139), and 1 with a trace of parakeratosis and cornification (No. 138).

Fractionated Radiation:

Series 139 a: (Femoral tumours).

Controls: A total of 5 (Nos. 75—79). Three squamous-cell carcinomas without parakeratosis or cornification, 1 with a trace of parakeratosis (No. 78), and 1 with a trace of cornification (No. 75).

Radiated tumours: (Radiation about once daily up to a total of 3250 r (200—200—350—350—350—450—400—450—500 r)): A total of 13 (Nos. 141—153). Ten squamous-cell carcinomas without parakeratosis or cornification, 2 with a trace of parakeratosis (Nos. 143 and 153) and 1 with a trace of parakeratosis and cornification (No. 150).

Series 144 a:

Controls: A total of 5 (Nos. 80—84). Two squamous-cell carcinomas without parakeratosis or cornification (Nos. 80 and 84), 1 with a trace of parakeratosis and cornification (No. 81), 1 with traces of

parakeratosis and moderate cornification in places (No. 82), and 1 with moderate parakeratosis and quite marked cornification in places (cornifying squamous-cell carcinoma) (No. 83, see Fig. 4). Four new sections from varying depths through the remainder of the block: In 2 sections a trace of cornification, in the other 2 no parakeratosis or cornification!

Radiated tumours: (Radiation once daily up to a total of 2600 r (300 — 350 — 400 — 450 — 500 — 600 r)): A total of 6 (Nos. 154—159). Five squamous-cell carcinomas without parakeratosis or cornification, 1 squamous cell carcinoma with marked parakeratosis and moderate cornification in places (parakeratotic and cornifying squamous-cell carcinoma) (No. 159). Four new sections through the remainder of the block: Squamous-cell carcinoma with slight parakeratosis and a trace of cornification in places!

Series 163 a:

Controls: The same as those used in the experiment with a single dose of 300 r.

Radiated tumours (300 r twice daily up to a total of 4800 r): A total of 9 (Nos. 160—168). Four squamous-cell carcinomas without parakeratosis or cornification (Nos. 160—162, 164), 2 with a trace of parakeratosis (Nos. 163 and 166), 1 with a trace of parakeratosis and cornification (No. 165, see Fig. 6), 1 with quite marked parakeratosis and cornification in places (No. 168), and 1 parakeratotic and cornifying squamous-cell carcinoma. Half the tumour was an undifferentiated squamous-cell carcinoma, the other half showed marked parakeratosis and cornification (No. 167, Fig. 7).

Series 163 b:

Controls: The same as those used in the experiment with a single dose of 300 r.

Radiated tumours (300 r twice daily up to a total of 4500 r): A total of 7 (Nos. 169—175). Six squamous-cell carcinomas without parakeratosis or cornification and 1 squamous-cell carcinoma with doubtful parakeratosis (No. 173).

Series 173 a:

Controls: The same as those used in the experiment with a single dose of 400 r.

Radiated tumours (400 r twice daily up to a total of 6000 r): A total of 17 (Nos. 176 —192). Eleven squamous-cell carcinomas without parakeratosis or cornification, 2 with a trace of parakeratosis (Nos. 178 and 186), 3 with a trace of cornification (Nos. 176, 177, and 180), and 1 with slight parakeratosis in places (No. 188).

(e) Evaluation of the radiation experiments:

Experiments with small single doses (100, 300, and 400 r) and

histological examination of the tumours at varying intervals within the first 48 hours: No increase in parakeratosis or cornification and no "orderly differentiation".

Single doses of 500, 1000, 1500, and 2000 r and histological examination from 1 to 8 days later: A few of the tumours exposed to large doses (1000—2000 r) exhibit a very slight increase in parakeratosis, irrespective of the time interval from the radiation. No definite increase in cornification and no "orderly differentiation".

Fractionated X-radiation of several experimental series with varying doses:

In series 139 a, receiving a total dose of 3250 r, no increase was discernible in parakeratosis or cornification.

In series 144 a, receiving a total dose of 2600 r, one tumour (No. 159) after receiving 2600 r exhibited marked parakeratosis and cornification in places. However, one of the controls (No. 83) showed an equally high degree of differentiation (see Fig. 4).

In series 163 a and b, receiving 300 r twice daily up to a total of 4800 and 4500 r respectively, two of the mice in series a treated with large doses (Nos. 167 and 168, see Fig. 7) showed quite marked parakeratosis and cornification. Series b derived from the same transplantation and submitted to exactly the same treatment hardly revealed any parakeratosis or cornification in any of the tumours. Taking both series as one, there was no definite increase, neither in parakeratosis nor in cornification, although a very slight, inconstant increase in both cannot be ruled out.

Series 173 a, comprising 17 tumours and receiving the heaviest radiation (400 r twice daily up to 6000 r) did not reveal any increase in parakeratosis or cornification!

The tumours treated with fractionated radiation did not either show signs of "orderly differentiation".

(f) Discussion: Those tumours which revealed more than a trace of parakeratosis or cornification were usually examined in several sections taken from various depths throughout the remainder of the block. Such examination revealed that the parakeratosis or cornification found do not extend throughout the tumour, being confined to small parts of it, apparently distributed at random. No difference was demonstrable between the parts of the tumour lying closest to the radiated skin and its other parts, and this was hardly to be expected, as the hard radiation used must be presumed to deliver approximately equal doses to all parts of the small, superficial tumours with which we are dealing.

Isolated parakeratosis and cornification, in places quite marked, as found in a few of the radiated tumours, is presumably due to chance, as it also occurred in some of the controls and does not seem to have been induced by any particular dosage.

On the other hand, it is probably not due to chance that some of the experiments revealed a small increase in the amount of parakeratosis in some of the tumours receiving massive single doses, and possibly also in a few cases submitted to fractionated radiation. It was not, however, possible to demonstrate a definite increase in the cornification, but a very slight, inconstant increase in the latter cannot be ruled out.

The increase in parakeratosis (and cornification) is so slight that it does not even lend itself to grading by the method of *Broders*, as most pathologists would probably allocate both the undifferentiated squamous-cell carcinomas and the slightly parakeratotic growths to Grade 3. It is my definite impression that the radiated tumours (see for instance Figs. 5 and 6) are no more "differentiated" than the untreated growths. They never exhibit the "regular and orderly" differentiation towards cornified cells which is an outstanding feature of squamous cells, and intercellular bridges were not observed anywhere. *It seems to me more likely that there is a certain selection of the (comparatively few) more differentiated cells in the tumour which are more radio-resistant than the (predominant) undifferentiated part of it which is more quickly affected by the radiation, degenerates more rapidly and is partly absorbed.* This theory is supported by the fact that the parakeratotic and cornified parts seen in tumours which have received fairly large doses (1000 r in one sitting or 2000 r fractionated) are nearly always in the process of breakdown, containing numerous leucocytes and macrophages. Theoretically, I had expected more parakeratosis and cornification in the radiated growths because of the difference in radio-sensitivity between undifferentiated and differentiated cells. Presumably the very limited increase is due to the marked capacity for absorption of a transplanted tumour on the part of the mouse organism. It must be remembered that each histological specimen from a tumour is an instantaneous photograph of extremely complicated and continuous processes. There is constant growth in the tumour (and more or less distinct attempts at differentiation) and tumour cells are constantly perishing and being absorbed, partly by humoral, partly by phagocytic activity. When X-radiation (in therapeutic doses as in the experiments under review) sets in, the growth of the tumour stops entirely or partly, and a large number of tumour cells degenerate in a brief space of time (less than 24 hours). If the radiations are continued, or if the single dose has been sufficiently large, the entire tumour may have disappeared within a week, a phenomenon which I have repeatedly observed. (Most of the latter experiments have been left out of this work, as they do not afford any elucidation of the differentiation).

This absorptive capacity (which is enormous in view of the body weight of the mouse) must also to a great extent apply to parakeratin and keratin as these formations would otherwise be found to be in-

creased in areas of the tumours undergoing spontaneous necrosis and connective-tissue organization. However, it would not seem unreasonable to me to assume that the absorption of horny material may be somewhat slower in tumours submitted to heavy radiation and in which large areas suddenly degenerate.

Another possible explanation is that the undifferentiated cells perish because of the radiation, whereas the more differentiated and therefore more radio-resistant cells go on differentiating (Regaud 1931, Lacassagne 1937). This explanation cannot be rejected entirely. Still, one would expect a more marked differentiation in the form of more numerous, large parakeratotic and cornified plaques and pearls, if the already somewhat differentiated cells went on differentiating after the radiation. The differentiation observed by me was, however, confined to quite small areas and only comprised a few cells. In fact, normal skin exhibits increased cornification following radiation. The explanation suggested is that the more differentiated cells are not destroyed, but proceed with their normal differentiation (see below). Worning (1937) has demonstrated the same process in the back hairs of guinea pigs following X-radiation.

The third possibility is that the X-rays should cause increased differentiation in the tumour cells, a theory advanced by several earlier workers, who have maintained that this increased differentiation was an important aspect of the effect of radiation. I think I am in a position to rule out this possibility. If X-rays did produce increased differentiation in a tumour, such differentiation would be expected to be far more marked and more uniformly distributed in the tumour following radiation than the faint signs of increased amounts of parakeratosis (and cornification) in a few parts of only a few tumours found in the experiments under review which revealed that all the other cells throughout the tumours only showed signs of marked radiation injury. The quite slight parakeratosis found cannot be regarded of any significance in the destruction of the growths, and the great majority of tumours in my material fail to show any hint of parakeratosis or cornification. Differentiation following radiation has often been reported, also during recent years. This is understandable, as the marked enlargement of the cells which may be combined with eosinophilia of the cytoplasm may easily lead one to interpret these phenomena as signs of increased differentiation (see Fig. 6).

Glücksmann, in 1941, reported findings of greatly increased differentiation following radiation of tumours which previously had revealed a tendency to differentiation. According to Glücksmann the criteria of differentiation are enlargement of the cells, polymorphism, and a deeper blue staining with Heidenhain's azan. He does not, however, look for parakeratosis or epithelial pearls which he does not consider to be a fit expression of the tendency to differentiation.

In Glücksmann's opinion a vacuolated, monstrous cell with a

partially destroyed nucleus should represent increased differentiation. I should not hesitate to interpret such a cell as one affected with severe degeneration, even when its cytoplasm is distinctly eosinophilic, if only it is pale and homogeneous. At any rate it cannot be interpreted as incipient cornification as such cells may be met with, for instance, following radiation of mammary carcinoma in which cornification is out of the question.

A cell which is greatly affected by radiation, enlarged, with a large hyperchromatic nucleus and an increased amount of cytoplasm, may perhaps have acquired more potential energy from the radiation, but this cannot be decided by the morphological investigations under review. There are, however, no morphological signs of increased differentiation in the sense of a more specialized cell function, but numerous morphological features characteristic of cellular degeneration.

In the case of some tumours I used *Heidenhain's* azan which stained the cytoplasm a reddish purple. The cytoplasm did not stain a deeper blue following radiation in my experiments.

Neither do I accept the view that parakeratosis and cornification should not be a fit expression of differentiation. In my opinion as well as that of most other pathologists, they afford the most distinct and most unmistakable signs of differentiation. It is a different matter altogether that their presence following radiation need not be tantamount to the radiation having exercised a differentiating influence. In the absence of parakeratosis and cornification (and intercellular bridges) in a squamous-cell carcinoma following radiation, it is in my opinion unnatural and wrong to talk about differentiation. Lastly, it is an outstanding feature of any cellular differentiation that the appearance and arrangement of the cells is "regular and orderly", and that I have never seen in the radiated tumours.

Neither have I found any signs of reduced differentiation in the radiated tumours, and the polymorphism which is a characteristic feature of the radiated tumours is never seen isolated, but always in connection with numerous signs of severe damage to the cells and is therefore naturally interpretable as an expression of cellular degeneration.

(3) Appendix.

Investigations into the Reaction of the Skin of Normal Mouse Tails to X-radiation:

These experiments were carried out with series 173 a: Squamous-cell carcinoma Aka.

The tumour was transplanted into the tails of Aka mice and the experimental mice received 400 r twice daily up to 9 days, a total of 6000 r. The technique was the usual one.

The normal skin covering the transplanted tumours was examined in 6 non-radiated controls (Nos. 56—61).

Stratum mucosum: The basal-cell layer (stratum cylindricum) often holds mitoses. The stratum spinosum consists of 3—5 layers of cells with distinct intercellular bridges. The stratum granulosum is not distinct, sometimes absent. Only in places where the epidermis is comparatively thick is there a well-developed layer with keratohyaline granules. The stratum lucidum is absent or only faintly visible.

The stratum corneum is rather thin, occupying on an average less than one-third of the epidermis, the stratum mucosum occupying the remaining two-thirds, but the thickness varies somewhat. At times it is absent artificially, due to the preparation of the specimen.

Series 473 a: (A total of 17 Aka mice). (Nos. 176—192).

After the first 4 radiations (i.e. after a total of 800, 1200, and 1600 r) there is a reduction in the number of mitotic figures and a slight enlargement of the cells. Apart from this no definite histological changes. Clinically, a transitory erythema of the skin on the tail is demonstrable following 800 r.

After 2000 and 2400 r have been delivered, there will be slight enlargement of the cells and nuclear degeneration, but the cornification remains unchanged. Following 2800 and 3200 r the enlargement of the nuclei and cytoplasm is moderate, and correspondingly there is moderate increase in the thickness of the entire epidermis, the stratum mucosum as well as the stratum corneum. A certain number of keratohyaline granules are seen.

Following 3600 r clinical examination shows a beginning, dry epidermitis, and histological examination reveals marked cellular degeneration and oedema of cells as well as horny layer. When 4000 and 4400 r have been delivered there are fewer keratohyaline granules, but *the cornification is now distinctly increased, relatively as well as absolutely, at the cost of the stratum mucosum which now consists of only 3 4—5 layers of cells as compared with 6—8 layers originally.*

Clinical examination (following 4400 r) shows a moderate, dry epidermitis.

Following 4800 and 5600 r the cornification increases, comprising now about half the epidermis. The stratum mucosum is constantly decreasing and even the most basal cells are flattened. There are hardly any keratohyaline granules. Below the basal membrane there is a good deal of leucocytes and oedematous fluid.

Following 6000 r the epidermitis is typically dry with areas of transition to moist epidermitis. *Four days after the last radiation (a total of 6000 r) nearly the entire epidermis has become cornified,* those basal cells which are still preserved exhibit marked degeneration, and there are no keratohyaline granules. The epidermis has often become detached from the corium and is partially necrotic.

Parakeratosis was not observed at any time.

Conclusion: Fractionated X-radiation of the normal skin of the tail stops cell growth (by inhibiting the mitotic activity) and produces degeneration of the cells (at a rather late juncture, as degeneration is still moderate following 2800 r). The cells undergo their normal differentiation in the usual way, and at a rather late juncture (following 3600 r) the cornification is seen to extend further basally than normal, and at the same time there will be clinical signs of a dry epidermitis.

After 6000 r there will be transition to moist epidermitis, and at this juncture the entire epidermis is cornified and it is desquamated, leaving the corium exposed. Accordingly, X-radiation causes degeneration of the basal cells, and during the period of the examinations new regeneration did not take place. On the other hand, *the cells were seen to undergo their normal*

differentiation (including the formation of keratohyaline granules) before they were desquamated.

This cornification in normal skin has been described by several earlier workers, *inter alia* by Clunet (1910), Nemenow (1925), and by Regaud (1931).

(4) Investigations into Histological Radiation Changes in General in Squamous-cell Carcinoma Aka.

Following X-radiation in *single doses of 100, 300, and 400 r*, allowing a latent period of about 2 hours, there is a doubtful to slight effect of the radiation in the form of a fall in the rate of mitotic activity of a few hours' duration. In about 12—24 hours 300 and 400 r are followed by slight enlargement of the cells and nuclei lasting for 12 hours. There are no other definite signs of the effect of radiation. The growth of the tumour remains completely unaffected.

Single doses of 500—2000 r.

500 r: After the lapse of 24 hours there will be a slight (doubtful) enlargement of the cells, but no other radiation changes. The growth of the tumour remains unchanged.

1000 r: After the lapse of 24 hours there are a few abnormal mitoses (in addition to a number of normal ones) and slight enlargement of the cells and some pyknotic nuclei. In 2—3 days there is also slight nuclear degeneration and a few multiple nuclei. In 4 days there are only a few mitotic figures, all distinctly abnormal, there is moderate enlargement of the cells and nuclei and karyolysis and karyorrhexis in several places. The nucleoli are often large and eosinophilic, and at times one may observe nuclear vacuoles. In 6—7 days the architecture of the tumour is in a state of marked dissolution, the enlargement of the cells increasing, the cytoplasm at times eosinophilic, and a number of the nuclei are monstrous; there are only a few mitotic figures (abnormal?) and there is beginning proliferation of fibroblasts. In 8 days only a mild effect of the radiation can be observed, but a few normal mitoses, i. e. beginning recurrence. The growth of the tumour is not definitely affected.

1500 r: The radiation changes appear somewhat earlier and are rather more marked. There is also a more distinct intercellular oedema and later considerable connective-tissue reaction with proliferation of fibroblasts; in addition, a number of leucocytes and lymphocytes and at times also plasma cells and histiocytes. As a rule the growth of the tumour has been arrested.

2000 r: The radiation changes are more intense, but otherwise as described above (see Fig. 5). The growth of the tumour has stopped or its size has diminished.

Fractionated X-radiation: (See Figs. 6 and 7). A typical reaction

was observed in series 173 a receiving 400 r twice daily up to a total of 15 sittings = 6000 r:

After the 2nd radiation a slight effect of the radiation is demonstrable in the form of slight enlargement of the cells and nuclei, blurring of the cell outlines and a few abnormal mitoses (in addition to a number of normal ones). The blood-vessels are slightly dilated. After 3—4 exposures there are fewer mitoses (usually abnormal), numerous pyknotic nuclei and a mild intercellular oedema. After 5—6 exposures the enlargement of the cells and the nuclear degeneration are moderate, and there is often karyorrhexis and karyolysis. There are a number of abnormal mitoses. After the 7th exposure a marked effect of the radiation is observed, predominated by large polymorphous, often monstrous and at times multiple nuclei with large nucleoli. There are only a few mitoses (all abnormal). After 8—9—10—11 exposures there are numerous leucocytes in the tumours which show increasing proliferation of fibroblasts. No mitoses. After 12—14 exposures the radiation changes are violent throughout the growth. There is often eosinophilic degeneration of the protoplasm. During the 1—4 days following the 15th exposure there are clusters of greatly affected cancer cells with enormous degeneration of the cells and nuclei in a vascular granulation tissue with marked proliferation of fibroblasts and beginning formation of collagen fibrils. In addition, there is a number of leucocytes and lymphocytes and a few plasma cells, histiocytes and foreign body giant cells. The nuclei are monstrous, hyperchromatic, often multiple, but without mitotic figures. The protoplasm is extremely swollen, often eosinophilic, at times also with large vacuoles. The original architecture of the tumour is completely obliterated. In the blood-vessels there is proliferation of the intima and hyaline degeneration of the media. Calcification was not observed (in this tumour).

The same reaction was observed in the other series treated with fractionated X-radiation, except for the fact that the reaction is somewhat fainter and sets in a little later because of the less intensive dosage.

In series 173 a the growth of the tumours was first inhibited, later arrested, and following the largest doses the tumours disappeared almost entirely in most cases, in 2 entirely. One mouse died with a recurrence one month after the last exposure. In the other "fractionated" series the growth was somewhat less affected, but still a few tumours disappeared completely. As the object of the experiments was to determine the differentiation, the great majority of the mice were killed before the tumour had a chance to disappear completely. The tumour must be considered comparatively radio-resistant, but in most cases it appears to yield entirely or practically to heavy fractionated radiation continued until a moist epidermitis sets in.

(5) Summary of Chapter IV.

Chapter IV describes X-radiation experiments with squamous-cell carcinoma Aka, a transplantable mouse carcinoma which originally was distinctly cornifying, but which in the later passages has been undifferentiated with sporadic signs of parakeratosis and cornification.

In all essentials the differentiation remains completely unchanged following radiation in single doses as well as following fractionated radiation. A few of the tumours exposed to large single doses, however, exhibit a quite slight increase in parakeratosis, irrespective of the interval from the radiation until the histological examination, but a definite increase in cornification is not demonstrable. When compared with the controls which now and then may exhibit considerable differentiation, the tumours treated with fractionated radiation do not reveal a definite increase in parakeratosis or cornification, although a quite slight, inconstant increase in both cannot be ruled out. In no case were there any signs of "regular and orderly differentiation" after the radiations.

The faint, inconstant increase in parakeratosis and doubtful increase in cornification following radiation cannot be interpreted as factors of any significance to the tumour destruction and cannot be taken as a sign of differentiation due to the radiation. The most reasonable explanation is that a selection takes place of the comparatively few, more differentiated tumour cells, whereas the undifferentiated, more radio-sensitive tumour cells degenerate in a higher degree after the radiation and are subsequently absorbed. Another (and less probable) explanation has been suggested, viz. that the undifferentiated, radio-sensitive cells should perish after the radiation, whereas the more differentiated and more radio-resistant ones should survive and go on differentiating. The experiments with normal skin of mice following fractionated radiation showed that this explanation must be accepted as far as the normal skin of mice is concerned.

The histological changes found in the 108 radiated tumours are described; they are all interpretable as an expression of cellular degeneration.

CHAPTER V

Skin Carcinoma

(Human Material).

(1) Material.

The material comprises 22 patients with skin carcinoma (Nos. 193—214). In 4 the carcinoma had its site on the trunk (the back in 2, the inguinal region in 1, the anococcygeal region in 1) and in the remaining cases it was on the head (the cheek in 5, the ear in 3, the nose in 3, the forehead in 3, the temporal region in 2, the pre-auricular region in 1, the scalp in 1). The patients ranged in age from 61 to 85 years. The growths chosen were rather large, so that several biopsies could be removed, as far as possible from the same part of the tumour.

On the basis of the biopsy specimens taken from the untreated tumours the material was grouped as follows:

Basal-cell carcinoma (<i>Krompecher</i> type)	11 (Nos. 193—203)
Basal-cell carcinoma (<i>Krompecher</i>) of the sweat gland type	2 (Nos. 204—205)
Atypical basal-cell carcinoma:	
In places large-celled carcinoma (mixed carcinoma?)	1 (No. 206)
Large-celled polymorphous	1 (No. 207)
Mixed carcinoma	1 (No. 208)
Squamous-cell carcinoma (without or with traces of parakeratosis and cornification)	6 (Nos. 209—214)

From 2 to 13 biopsy specimens were removed from each patient, 110 specimens in all. Most of them were excision biopsies and in a few cases the entire tumour was available.

(2) Radiation Technique.

The patients were treated partly with fractionated X-radiation with rays of different qualities, partly with interstitial radiation (needling) a few with both.

Hard X-radiation was administered with a Thoraeus filter, 180 kv., 6 ma., half-value layer 1.75 mm. Cu., focal-skin distance 50 cm., intensity 8.4 r/min. *Rather soft X-radiation* was administered with a filter of 3 mm. Al., 100 kv., 2 ma., half-value layer 3 mm. Al., focal-

skin distance 40 cm., intensity 9.1 r/min. Lastly, *contact therapy with soft rays*, Monopan 60 kv., 4 ma., half-value layer 2.2 mm. Al., focal-skin distance 3—5—7 cm. and intensity 408 r/min., 147 r/min. and 74 r/min. respectively.

The *tumour dose* in the surface delivered by hard as well as rather soft X-radiation was calculated on the basis of the tables in *Glasser's* paper (1944), that delivered by contact therapy according to *Quimby, Marinelli & Farrow* (1938).

Interstitial radiation was performed with needles holding 9—10 mg. of radium ($\frac{1}{2}$ mm. platinum filter) applied for 4—5 hours.

In the radium-treated tumours the *tumour dose* was calculated in a plane 3 mm. distant from the axes of the radium needles (according to *Ebenius* 1943) and converted into r. The tumour dose was calculated partly at the ends of the active portion of the most peripheral needle, partly in the centre of the tumour, the two tumour doses being expressed as minimum and maximum respectively.

All the calculations are approximate, and the tumour dose in the surface may presumably differ considerably from the computed values, especially in the tumours treated with interstitial radiation.

(3) Radiation Experiments.

Two tumours (Nos. 197 and 209) were treated with hard X-radiation, the tumour dose being about 3500 r in both cases. Four tumours (Nos. 206, 207, 211 and 214) received X-radiation with a filter of 3 mm. Al., 100 kv., the tumour dose varying from 4600 to 6200 r. Eight tumours received contact X-ray therapy (Nos. 194, 199, 202—205, 210 and 213), the tumour dose varying from 4700 to 8800 r. Two tumours (Nos. 195 and 200) received both X-radiation with a filter of 3 mm. Al., 100 kv. and contact therapy, the tumour dose being 5200 and 4000 r respectively. Interstitial radiation was employed in 5 cases (Nos. 193, 196, 198, 201 and 208), the minimum tumour dose varying from 2000 to 3000 r, maximum tumour dose from 3000 to 5100 r. Because of recurrence 1 tumour (No. 212) received X-radiation partly with a Thoraues filter (tumour dose 2700 r) partly with 3 mm. Al., 100 kv. (tumour dose 9700 r) and later 2 interstitial radium treatments with a minimum tumour dose of 2800 and 2300 r respectively and a maximum tumour dose of 4900 and 3400 r respectively.

(4) Investigations into Alterations in Differentiation

Following X-rays and Radium.

a. Differentiation and degree of malignancy. The criteria used for the evaluation of the tendency to differentiation in the skin carcinomas were those set up in connection with the squamous-cell mouse

carcinoma. Signs of differentiation were the following: Intercellular bridges (prickle-cell elements), parakeratosis and cornification (perhaps with keratohyaline granules), glandular imitation and structures resembling hair follicles. On the other hand, polymorphism which is of great significance in the evaluation of the biological malignancy, was not included in the criteria. From a morphological point of view there is not much difficulty in ascertaining the presence or absence of these signs of differentiation, and skin carcinomas are therefore suitable for studies of this nature. On the other hand, it is extremely difficult to judge the significance of the various signs of differentiation to the biological and clinical malignancy, as skin carcinomas are divisible into two large groups, basal-cell and squamous-cell carcinomas which cannot be classified or compared histogenetically.

The findings are most clearly interpretable in squamous-cell carcinoma which shows a degree of differentiation ranging from the (rather uncommon) undifferentiated, polymorphous tumours, perhaps with a certain tendency to differentiation into prickle-cells, but without parakeratosis or cornification (corresponding to *Broders'* Grade 4—3) to tumours showing more or less marked prickle-cell elements, parakeratosis and (or) cornification. In the highly differentiated varieties there will be rather »orderly« differentiation, several keratohyaline granules and abundant cornification (corresponding to *Broders'* Grade 1). The most highly differentiated epithelial tumour, papilloma, may be included in this category and classified as a Grade 0. Squamous-cell carcinoma thus displays varying degrees of differentiation in analogy with carcinoma of the oral cavity and the oesophagus, and it is generally agreed that the degree of differentiation found in these skin tumours is an adequate expression of their biological malignancy and radio-sensitivity (which are in reverse proportion to each other).

Much more difficulty is encountered in basal-cell carcinoma of the skin (*Krompecher's* tumour). It is made up of islets or strands of (usually) small, hyperchromatic, slightly polymorphous cells, bearing distinct marks of being derived from basal cells. It cannot, however, be seen from the appearance of the cells (regardless of their tendency to differentiation, if any) whether they arise in the stratum cylindricum of the epidermis or in the anlagen of hairs, sebaceous glands or sweat glands (emphasized *inter alia* by *Broders & MacCarty* (1918) and *Eller* (1939)), as basal epithelial cells normally are capable of forming epidermis as well as hair follicles, sebaceous glands, and sweat glands.

In certain cases a basal-cell carcinoma shows signs of differentiation in the form of atypical glands (sweat or sebaceous glands), structures resembling hair follicles or a few scattered parakeratotic or horny pearls without otherwise changing its character as a basal-cell carcinoma.

Basal-cell carcinoma is generally benign and of slow growth. It responds well to radiation (with the exception of the sweat gland tumours) and seldom recurs after adequate radiotherapy or deep excision. Several workers doubt whether basal-cell carcinoma can at all be called a malignant tumour (e. g. *McCarthy* (1931)), and as emphasized *inter alia* by *Tailhefer & Courtial* (1943) typical basal-cell carcinoma has never been known to metastasize, unlike squamous-cell carcinoma and the atypical varieties. In long-standing untreated cases it may, however, often invade the cartilage or bone, or else it may acquire a monstrous size and become considerably more radio-resistant. In such cases the tumour behaves exactly like malignant growths and its clinical malignancy is marked. The usual benign course and cure following radiotherapy is probably explicable by the superficial growth of basal-cell tumours and the absence of any tendency to invade blood-vessels and lymphatics (*Lacassagne* 1936).

According to recently advanced views (*Roussy, Laborde & Huguenin* (1943)) basal-cell carcinoma of the skin must still be considered more radio-sensitive than squamous-cell carcinoma, but the difference is stated to be slight.

As long as the tumours retain their basal-cell character (apart from the above-mentioned sparse, scattered tendency to differentiation) they must be regarded comparatively benign. However, as soon as they become atypical, polymorphous (corresponding to "the intermediate type") or exhibit signs of differentiation into prickle-cells (mixed carcinoma) with intercellular bridges, large, flat epithelial cells and perhaps large amounts of parakeratosis or cornification, they have been found to show greater clinical malignancy (with a tendency to recurrences and metastases and probably also higher radio-resistance) on a level with squamous-cell carcinoma. This cannot be explained on the basis of the ordinary views on differentiation in new growths and its influence on the biological malignancy, as it is a question of a tumour (basal-cell carcinoma) acquiring higher differentiation (mixed carcinoma, squamous-cell carcinoma) and at the same time greater biological (and clinical) malignancy. (On the other hand, the alteration in radio-sensitivity appears to be proportional to the degree of differentiation). For the time being we can only advance the unsatisfactory explanation that there has been a change of one type of tumour into another, and the two types are therefore not directly comparable. Regrettably, it is sometimes difficult to decide whether one is faced with a basal-cell carcinoma with scattered (unimportant) signs of differentiation or with one of the atypical varieties (mixed carcinoma) with a far more serious prognosis. It seems as if recurrences of basal-cell carcinoma have a tendency to become mixed or squamous-cell carcinomas (*Körbl*, 1912). The same applies to the rare cases of metastases from basal-cell car-

cinomas described. These difficulties are also reflected in the small material under review which showed the same tendency.

b. Histological examination. The differentiation in the material under discussion remained unchanged during and after the treatment (see Figs. 8—11). Only Case 198 in which the specimen from the untreated tumour showed a typical basal-cell carcinoma, 3 of the 4 biopsies examined later showed a couple of small parakeratotic pearls and 1 also a horny pearl. In addition, Case 208 (mixed carcinoma) revealed a slight increase in the number of horny pearls. On the other hand, biopsy from the untreated tumour was sometimes found to contain more epithelial pearls or other signs of differentiation than the specimens removed during treatment. Presumably these findings have been due to change because the differentiation varies in the different parts of the growths and because the biopsy specimens were rather small. No relation was found between the quality or intensity of radiation and the differentiation of the growths.

Accordingly, there is no evidence that X-radiation or radium should cause alterations in the differentiation of human skin carcinoma. There was not either any relative increase in parakeratosis or cornification, mild signs of which had been observed in a few of the animal experiments with squamous-cell carcinoma. Aka. In skin carcinoma showing marked effects of radiation it may at times be difficult to discern the neoplastic tissue from the tongue-shaped masses extending down into the dermis from the normal surface epithelium which may give the appearance of islets of highly degenerated epithelial cells with central horny or parakeratotic pearls. Misinterpretations of this nature perhaps account for several of the cases of cornification following radiation described in the literature.

Four of the tumours recurred several years later (and a few failed to yield completely to the treatment). The recurrence in No. 193 was made up of larger cells and was more atypical than the original basal-cell growth, No. 198 was still a typical basal-cell tumour, whereas Nos. 200 and 204 recurred as mixed carcinomas although the original growths had been basal-cell carcinoma with one parakeratotic pearl and basal-cell carcinoma of the sweat gland type respectively.

Hepatic metastases were observed in 1 case (No. 206). Biopsy from the untreated tumour had shown basal-cell carcinoma, large-celled in places and with a few parakeratotic and horny pearls (atypical, mixed carcinoma?). The hepatic metastasis was also predominantly a basal-cell growth, but with differentiation in a few places to prickle-cell carcinoma with a few parakeratotic and horny pearls. Thus, the tumour cannot be regarded as having been a typical basal-cell carcinoma.

The patients have not been followed up for 5 years in all cases, and the material does not allow of conclusions as regards the relation between the histological appearance of the tumour, the histological radiation reaction, and the effectiveness of the treatment.

(5) Investigations into Histological Radiation Changes in General in Skin Carcinoma.

The radiation changes demonstrable by histological examination do not differ from those observed in the squamous-cell mouse carcinoma, and the changes caused by radium and X-rays were identical, regardless of the quality of the rays. Histologically different types of growth also respond in the same way in all essentials. Still, it is my impression that the cellular radiation changes observed are less pronounced in basal-cell carcinoma than in squamous-cell carcinoma. This difference applies chiefly to the sweat gland tumours in which the enlargement of the cells, nuclear degeneration etc. was found to be slight. This does not, however, justify the conclusion that basal-cell carcinoma is less radio-sensitive than squamous-cell carcinoma, as highly radio-sensitive tissues (for instance leukaemic tumours) are destroyed so rapidly that the radiation changes demonstrable upon histological examination will be quite mild. Some of the basal-cell growths (e. g. Nos. 202 and 203) thus failed to show much radiation reaction histologically in spite of a satisfactory clinical effect.

After about 500–1000 r the *X-radiated tumours* show a slight effect of the radiation (in the form of few or no mitotic figures, some blurring of the cell outlines and slight enlargement of nuclei and cells). Following about 1500–2500–3000 r the effect is moderate (moderate enlargement of cells and nuclei, nuclear degeneration, perhaps karyorrhexis and karyolysis, and a few abnormal mitotic figures; at times there will be mild eosinophilic protoplasmic degeneration). At a later stage of fractionated treatment the changes become more pronounced (see Fig. 9), often showing comparatively large, polymorphous, hyperchromatic nuclei with large nucleoli, sometimes multiple nuclei. There is a simultaneous increase in the amount of connective tissue and the number of leucocytes and lymphocytes, at times also of plasma cells. Towards the end of the treatment and during the first weeks (or sometimes months) after one may find a granulation tissue sprinkled with islets of highly degenerated tumour tissue, and sometimes calcified areas in the stroma or tumour tissue (see Fig. 11). The blood-vessels show the changes described above.

Radium treatment is followed by the same changes which usually are demonstrable in 24 hours. In 3–5–7 days the effects of the radiation are moderate, during the subsequent days marked, whereupon they appear to subside slowly, but neoplastic cells have been observed as late as 1 month after interstitial radiation. The intensity of the radiation changes appears to be about the same as following X-radiation with the dosage employed with the only difference that the histological changes appear rather sooner after radium treatment.

In those tumours from which several biopsies were removed from the same part, the tumour cells were often found to be surrounded by

abundant granulation tissue at an early juncture and seemed to degenerate somewhat earlier and more violently than in other cases. This does not, however, essentially affect the course of the radiation reactions or the differentiation.

(6) Summary of Chapter V.

A total of 22 skin carcinomas from patients treated at the Radium Centre were examined histologically before, during and after treatment with X-rays or radium. Of these tumours 13 were basal-cell carcinomas (*Krompecher* type), and of them 2 were of the sweat gland type, 3 basal-cell growths, but atypical, and 6 squamous-cell carcinomas without or with parakeratosis and cornification. The histological signs of differentiation in skin carcinoma are easily demonstrable, but the differentiation does not accord with the biological (and clinical) malignancy, and the two types, basal-cell and squamous-cell carcinoma, are not directly comparable. The differentiation remained unchanged after the radiotherapy with the exception that a few cases exhibited some increase or some decrease in the number of parakeratotic or horny pearls as compared with the specimens from the untreated tumours. This is presumably attributable to chance, partly due to the rather varying differentiation in the different parts of the growths, partly to the small size of the biopsies. No relative increase was found in parakeratosis or cornification.

It is emphasized that in skin carcinoma showing marked radiation reaction neoplastic tissue may be difficult to discern from tongue-shaped masses extending down into the dermis from normal epithelium. It is suggested that this may account for a number of previously reported cases of cornification following radiation.

The histological radiation changes in skin carcinoma are described. The intensity of such reactions was comparatively slight in some of the basal-cell carcinomas in spite of a satisfactory clinical effect. The cellular changes correspond to those observed in squamous-cell carcinoma Aka.

CHAPTER VI.

Carcinoma of the Cervix Uteri

(Human Material).

(1) Material.

The material comprises biopsies taken from 31 patients with cervical carcinoma (Nos. 215—245). The patients chosen had medium-sized, exophytic, non-necrotic tumours on the portio vaginalis where serial biopsies could be removed, as far as possible from the same part of the tumour. Adenocarcinomas were left out.

All the biopsies were taken with a biopsy forceps. From 3 to 8 specimens were removed from each patient, a total of 129 specimens.

(2) Radiation Technique.

The treatment was that customary at the Radium Centre, consisting in 26 cases in combined X-rays and radium, in 2 cases in X-ray treatment only, and in 3 in radium treatment only.

In more than half the cases (16 to be exact) the method was *X-ray rotation therapy* with a rotating tube, 400 r twice daily to two fields, an anterior and a posterior field, each comprising 180° . The radiations were administered with a filter of $\frac{1}{2}$ mm. Cu., 185 kv., 15 ma., half-value layer 0.9 mm. Cu., focal-skin distance 35 cm., area of field on the skin about 6×8 cm., intensity 84.8 r/min. The total dose aimed at was about 18,000 r combined with two radium applications, the first after 12,000 r and the second after the conclusion of the X-ray course about 10 days later.

The remaining 12 X-radiated cases received *fractionated X-radiation*, 200 r twice daily to 8 fixed fields. The area of the fields varies from 10×10 to $12\frac{1}{2} \times 12\frac{1}{2}$ or 10×15 cm. As a rule the technique was $\frac{1}{2}$ mm. of Cu., 180 kv., 6 ma., half-value layer 0.9 mm. Cu., focal-skin distance 40 cm., intensity 30 r/min. In a few instances the same quality of rays was used as in the rotation therapy or with the Siemens Bomb, $\frac{1}{2}$ mm. Cu., maximum 193 kv., 20 ma., half-value layer 0.85 mm. Cu., intensity 49 r/min. In fractionated radiation the aim was 60 exposures = 12,000 r combined with 2 radium applications, the first following 8000 r and the second upon conclusion of the X-ray course about 10 days later. The entire treatment takes rather more than a month. In

a few instances the radium applications were given at some other stages of the X-ray course.

Radium application (combined with X-radiation) usually means the application of 30 mc. of radium emanation (screened by 1 mm. platinum) into the cervical canal and 50 mg. of radium (screened by 3 mm. lead) into the vagina below the portio. With a radiation period of 20 hours the dose is 600 mch. (millicurie hours) + 1000 mgh. (milligram hours) in each sitting. If radium alone is used without X-radiation, the dose is 1400 mch. + 2000 mgh. in each sitting.

The tumour dose in the tumours of the portio vaginalis following 18,000 r in rotation therapy has been calculated after *Glasser* (1944) to be about 6000 r, provided that the fields were stationary. Following fractionated X-radiation with 12,000 r the tumour dose has been measured by *Anton Jensen* (1945) to be about 3500 r.

Following radium application it was attempted to calculate the tumour dose at a distance of 1 cm. from the radium source. When radium alone is used, the tumour dose delivered in both sittings has been calculated to total about 40,500 r. Following radium combined with X-rays the tumour dose delivered in both radium applications has been calculated to be about 19,500 r.

Hence, the aggregate tumour dose at a distance of 1 cm. from the radium source is about 25,500 r following rotation therapy combined with radium and about 23,000 r following fractionated X-radiation combined with radium.

It must be pointed out that these figures are based on an estimate. Besides, it is impossible to state the exact distance from the radium applicators to the parts whence the biopsies were removed, and therefore there may be wide variations in the tumour doses in the specimens examined.

(3) Investigations into Alterations in Differentiation Following X-rays and Radium.

Histologically the material has been classified on the basis of the biopsies from the untreated growths into:

- | | |
|---|----|
| 1. Squamous-cell carcinoma. Undifferentiated, without or with traces of parakeratosis (Nos. 215—221). | 7 |
| 2. Squamous-cell carcinoma. Rather undifferentiated without or with traces of parakeratosis (Nos. 222—231). | 10 |
| 3. Parakeratotic squamous-cell carcinoma (Nos. 232—244). | 13 |
| 4. Parakeratotic and cornifying squamous-cell carcinoma (No. 245). | 1 |

The criteria of differentiation during the radiations were partly parakeratosis (and cornification) which are easily demonstrable in spite of the marked effects of radiation on the cells, partly the more

or less marked tendency of the growths to exhibit squamous epithelium ("orderly" differentiation), whereas polymorphism following the radiations has not been attributed with any importance. Intercellular bridges and keratohyaline granules were not observed in any case and actual cornification in only one.

The 7 cases of undifferentiated squamous-cell carcinoma without or with traces of parakeratosis (Nos. 215—221) did not display any alteration in the differentiation during the radiations. Such alteration could not either be found in the 6 cases of rather undifferentiated carcinoma without parakeratosis (Nos. 222—227) (see Figs. 12 and 13), whereas only 1 (No. 230) of the 4 rather undifferentiated squamous-cell carcinomas with traces of parakeratosis remained unchanged and 1 (No. 228) revealed a slight increase in parakeratosis. In the remaining 2 cases (Nos. 229 and 231) parakeratosis was not demonstrable during the radiations. Otherwise, the differentiation remained unchanged in all the cases.

Among the 13 cases of parakeratotic squamous-cell carcinoma (Nos. 232—244) the differentiation remained unchanged in 6, whereas 6 (Nos. 233, 235, 237, 238, 243, 244) showed less or no demonstrable parakeratosis during the treatments. These findings were, however, in most instances made in the last biopsies which were made up of granulation tissue with relatively few cancer cells, showing marked effects of radiation. It strikes one, however, that a relative accumulation of parakeratosis *does not* take place in these parakeratotic tumours.

Case 234, a squamous-cell carcinoma with excessive parakeratosis centrally in the islets of tumour cells (Fig. 14) but few parakeratotic pearls and no actual cornification, behaved in a way extremely different from the others during radiation (fractionated X-radiation combined with radium as usual). In the first 2 biopsies during treatment (following 2000 and 4000 r) the differentiation was found to be practically unchanged, perhaps with a slight increase in parakeratosis; the tumour cells showed marked effects of radiation (Fig. 15). In biopsies following 6000 and 8000 r, however, the central parakeratotic masses had increased, whereas the peripheral area of non-parakeratotic tumour cells had narrowed; as previously, there were only a few parakeratotic pearls and no cornification. The next 3 biopsies (following 9600 r, 12,000 r and 16 days after the termination of the X-radiation (12,000 r) which is 16 and 2 days respectively after the 1st and 2nd radium application) were made up of granulation tissue with large masses of parakeratotic substance which was being absorbed (Fig. 16), but there were no peripheral areas of tumour cells, and there were still only a few parakeratotic pearls and no cornification.

In this instance there is no doubt that the pre-existing excessive parakeratosis increased during the treatment, but there was no increase in parakeratotic pearls, no cornification or "orderly" differentiation.

In the only "actually" cornifying squamous-cell carcinoma (No. 245) the differentiation remained completely unchanged during treatment.

Among all 31 cases the differentiation remained completely unchanged in 10, whereas the amount of parakeratosis in 8 of the tumours had decreased or was undemonstrable after radiation, especially in the last biopsies which only revealed a few cells, showing marked effects of the radiation, scattered in a granulation tissue. Only two cases exhibited increased parakeratosis, one in a mild degree (No. 228), whereas the other one (No. 234) showed considerable relative increase in parakeratosis, but no signs of an "orderly" increased differentiation as compared with the untreated tumour. There was nothing else to indicate increased differentiation or an increase in parakeratosis or cornification explicable in some other way. Neither was there any evidence of a decreased differentiation following radiation, as the demonstration of a comparatively slight decrease in the amount of parakeratosis observed in a few cases probably was due to chance. No relation could be found between the nature or intensity of the radiations and the differentiation of the tumours.

It is, however, apparent from Case 234 that in certain instances a considerable increase in parakeratosis *may* occur during radiation as reported by several earlier workers. The phenomenon resembles the findings following radiation of normal mouse (and human) skin. It is perhaps explicable by a radiation-induced arrest in cell division, whereas the differentiation (high in these cases) continues unchanged, until all the cells have become parakeratotic, lying as large parakeratotic horn-like masses in order to be gradually absorbed.

Another explanation of the increased parakeratosis is that the parakeratotic elements degenerate and are absorbed rather more slowly than the non-parakeratotic cells which perish and disappear more rapidly. This would result in a relative increase in parakeratotic cells. Moreover, the remaining parakeratotic masses will be swollen owing to imbibition of water. In view of the fact that the tumour, which originally was as large as a closed fist, subsided almost entirely in the course of the month of treatment, and that the biopsies revealing increased parakeratosis were taken from the small remnant of the growth, I think that the most probable and most satisfactory explanation of the increase in parakeratosis is such a relative accumulation of parakeratosis because of a slower absorption.

In my opinion the reason why this tumour behaved so differently is most probably that its tendency to parakeratosis was exceedingly marked before the radiations. The therapy did not in any respect differ from the normal procedure and the tumour responded satisfactorily.

As far as I can see, the third possibility, i. e. that there should have been an increase in differentiation during treatment, can be ruled out, as the appearance of the tumour did not at any time after

the radiations suggest a more "orderly" differentiation and as it failed to exhibit more parakeratotic pearls, traces of intercellular bridges, or actual cornification.

Twelve patients are alive without recurrence (follow-up period, however, only more than 5 years in 2 cases), 1 died from intercurrent disease, and 18 had recurrences, leading to death in 12. Among the recurrences, microscopic examination is available in 3: Nos. 220, 223, and 224. This revealed a differentiation unchanged in all essentials. However, Case 224 (a rather undifferentiated squamous-cell carcinoma without parakeratosis) was more polymorphous.

No definite relation has been observed between the degree of differentiation, the degree of the histological radiation reaction and the end result. The material is, however, too small, too inhomogeneous, and the follow-up too brief to allow of decisive conclusions in this respect.

(4) Investigations into Histological Radiation Changes in General in Cervical Carcinoma.

The histological radiation changes in the 31 cases of cervical carcinoma do not differ essentially from those observed in squamous-cell carcinoma Aka and skin carcinoma. The findings are the same following X-radiation and radium with the only exception that the radiation changes appear sooner and are of a more violent nature following radium application as must be expected in view of the very large tumour doses in the superficial parts of the cervical tumours, the very parts used for biopsy.

After X-radiation with about 1000—1500 r the specimens show moderate effects of the radiation (cf. the description of the histological radiation changes in squamous-cell carcinoma Aka in Chapter IV, Section 4). As the dose is increased, the radiation changes quickly become more marked, the biopsies often showing pronounced eosinophilic protoplasmic degeneration which nearly always is easily discernible from parakeratotic masses. Other changes observed at an early juncture are dense infiltration with leucocytes, plasma cells and lymphocytes as well as hyperæmia and oedema.

When 5000—8000 r have been delivered the radiation changes are well pronounced with marked proliferation of fibroblasts and later collagen connective tissue. At the same time there will be distinct vascular changes in the form of proliferation of the intima, hyaline degeneration of the media and thrombosis.

Following 8000 r (fractionated) — 12,000 r (rotation therapy) there are usually islets of neoplastic cells showing marked effects of radiation: greatly degenerated nuclei and a swollen eosinophilic protoplasm in an oedematous granulation tissue where eosinophilic leucocytes predominate.

At a later stage of the radiation it may be difficult to point out any tumour cells, although quite large remnants of the tumour may be demonstrable clinically. This applies particularly to radium-treated tumours which show marked effects of the radiation in 3—4—5 days. These effects, however, subside gradually, but months after the radium treatment one may observe distinct radiation changes in the tumour cells.

At a later stage of the treatment calcification is seen, partly in the tumour cells, partly in the connective-tissue stroma.

(5) Summary of Chapter VI.

Chapter VI deals with the histological changes observed in 31 cases of human carcinoma of the cervix uteri following therapeutic irradiation with X-rays and radium. More than half the cases were undifferentiated or rather undifferentiated squamous-cell carcinomas without or with a trace of parakeratosis, whereas the remainder were rather highly differentiated squamous-cell parakeratotic carcinomas, cornifying in one case.

The amount of parakeratosis and the differentiation on the whole remained unchanged after the treatment except for negligible fluctuations, and a relative increase in parakeratosis was only demonstrable in one case (No. 234) with pre-existing excessive parakeratosis. This tumour did not either exhibit "orderly" differentiation, intercellular bridges or actual cornification. The most probable explanation of the increased parakeratosis is a relative accumulation of parakeratotic cells owing to a somewhat slower degeneration and absorption as compared with non-parakeratotic cells. It cannot, however, be excluded that the increase in the amount of parakeratosis *may* be due to radiation-induced arrest of cell division, whereas the differentiation continues until all the cells have become parakeratotic.

A description is given of the histological changes observed in the radiated uterine cancers. The findings do not differ essentially from those encountered in mouse and human squamous-cell carcinomas.

CHAPTER VII.

Mammary Carcinoma Dlb.

(Transplantation Experiments with Mice).

(1) Material.

The experiments were performed with a transplanted tumour "Th. II" into Dlb mice (a subline of Little's dilute brown (dba)). In 1942 Dr. V. *Thayssen* at the Radium Centre transplanted a spontaneous mammary tumour into Dlb mice in which it took in about 90 per cent. and led to death in the course of a couple of months, often with pulmonary metastases.

Microscopical examination of untreated tumours (see Fig. 17) shows a rather uniform picture of a solid undifferentiated carcinoma without glandular imitations, but often with small "pseudo-acinous" cavities filled with fluid between the neoplastic cells. The cells are rather large, distinctly epithelial, the nuclei round or oval, moderately rich in chromatin and with a distinct nucleolus. There are numerous normal or slightly abnormal mitotic figures. The protoplasm is pale, rather sparse and rather ill-defined, holding no secretion bodies or vacuoles. There is only slight polymorphism and hyperchromatism, but often pyknotic and necrotic areas. As a rule the connective-tissue stroma is sparse.

Glandular imitations were not found in any of the untreated tumours (transplanted in most cases to the tails), but often "pseudo-acini". Absolutely definite glandular imitations were encountered in 3 of 12 pulmonary metastases (Nos. 283, 17132 II, No. 285, 17137 II (both of Series 321, controls) and No. 366, 17184 (Series 323 a)) and in an intra-peritoneal metastasis (No. 285, 17137 III, Series 321, control) (see Fig. 18). This shows that in certain circumstances the tumour may differentiate into an adenocarcinoma.

Tumours transplanted into mouse tails are often rather different from growths transplanted into the axilla and from secondary growths, the neoplastic cells in the tail tumours often being somewhat smaller, more spindle-shaped, and the number of mitotic figures reduced, presumably due to the more difficult nutritive conditions and the pressure of the taut skin.

If the transplant does not take or if the take is poor, there will be few or no mitoses, small, often pyknotic cells, more necrotic areas and a more ample connective-tissue stroma.

(2) Radiation Experiments.

The X-radiation experiments were carried out exclusively with tumours transplanted into the tail. The technique was the usual one.

Experiments:

Single dose of 25 r: (Nos. 289—298, Series 325 i). A total of 10 mice, killed 1 hour to 6 days after the exposure.

Single dose of 300 r: (Nos. 299—308, Series 325 b). A total of 10 mice, killed from 1½ to 24 hours after the exposure.

Single dose of 500 r: (Nos. 309—318, Series 325 a). A total of 10 mice, killed 1—16 days after the exposure.

Single dose of 1000 r: (Nos. 319—328, Series 323 b). A total of 10 mice, killed 22 hours to 21 days after the exposure.

Single dose of 1500 r: (Nos. 329—337, Series 321 b). A total of 9 mice, killed 2 to 69 days after the exposure.

Single dose of 2000 r: (Nos. 338—347, Series 321 a). A total of 10 mice, killed 1 to 19 days after the exposure.

Fractionated X-radiation, 400 r twice daily, up to a total of 400 r $\times$ 13 = 5200 r: (Nos. 348—365, Series 323 a). A total of $20 \div 2 = 18$ mice, killed 20 hours after the first exposure to 10 days after the last exposure.

A total of 77 X-radiated tumours and 43 controls (Nos. 246—288) were examined. In addition, there are 14 untreated controls in the form of metastases, mostly in the lungs, partly in untreated, partly in radiated mice.

(3) Investigations into Alterations in Differentiation Following X-radiation.

Histological examination of the 77 X-radiated transplanted tumours did not in any case reveal higher differentiation in the form of attempts at glandular imitations, and the differentiation appears to correspond exactly (Figs. 19—20) to that found in the 43 untreated transplanted tumours which also were undifferentiated (Fig. 17). As mentioned above, however, 3 of 12 pulmonary metastases and 1 intraperitoneal metastasis (Fig. 18) showed unmistakable signs of differentiation into an adenocarcinoma. One of the 3 pulmonary metastases was in a mouse (No. 366) which had received $400 \text{ r} \times 13 = 5200 \text{ r}$ 20 days before it was killed, but the lungs cannot have received radiation because of the protective lead covering. The other differentiated secondaries were encountered among untreated controls. The fact that the tumours exhibiting higher differentiation were secondary is perhaps due to chance or perhaps to the altered conditions of growth.

(4) Investigations into Histological Radiation Changes in General in Mammary Carcinoma Dlb.

The histological radiation changes following X-radiation of mammary carcinoma Dlb are in all essentials of the same appearance as the findings in the tumours reported above, and the reader is therefore referred to the description of radiation changes in squamous-cell carcinoma Aka in Chapter IV, Section 4.

The tumour is considerably radio-resistant and only a few of the growths receiving large fractionated doses (5200 r) disappeared entirely. In spite of this radio-resistance, large doses may be followed by marked radiation changes (see Fig. 19 and 20) which, however, are less pronounced than in squamous-cell carcinoma Aka. In some of the tumours receiving large doses the protoplasm was found to be slightly eosinophilic, though not by far to the extent seen in squamous-cell carcinoma. Metaplasia to squamous epithelium was not observed.

(5) Summary of Chapter VII.

An undifferentiated solid mammary carcinoma transplanted into mice was used for X-radiation experiments. The differentiation remained low in the 77 radiated tail tumours, although a few of the untreated metastases exhibited a definite capacity for differentiation in the form of atypical glandular imitations.

The histological findings following X-radiation were of the same appearance as in the tumours described above.

CHAPTER VIII.

Mammary Carcinoma

(Human Material).

(1) Material.

The material comprises biopsies from 29 patients with mammary carcinoma (Nos. 367—395), selected at random among the patients treated at the Radium Centre. Biopsy (excision biopsy or punch biopsy*) was removed in all cases prior to treatment. (Cases 368 and 391 had, however, received 150 r 1 and 2 days respectively before the removal of the biopsy). During or after X-radiation at least 2 and usually more biopsies were taken. In 20 cases histological examination could also be made of the removed breasts, in some of them also of metastases to the axillary lymph nodes.

(2) Radiation Technique.

The technique used was the one customary at the Radium Centre in pre-operative radiation: $\frac{1}{2}$ mm. Cu., 180 kv., 6 ma., half-value layer 0.9 mm. Cu., focal-skin distance 40 cm., intensity 30 r/min. The radiations to the supraclavicular field were, however, given with a filter of 3 mm. Al at a focal-skin distance of 50 cm. A total of 4 fields were radiated:

- (1) Tangential field on medial aspect of breast 10×15 cm.
- (2) Tangential field on lateral aspect of breast 10×15 cm.
- (3) Axillary field about 8×10 cm.
- (4) Supraclavicular field (varies somewhat in size).

The dose delivered to each field is $150 \text{ r} \times 14 = 2100 \text{ r}$, the axillary field, however, receiving only $150 \text{ r} \times 12 = 1800 \text{ r}$. Two fields are radiated daily, and the entire course lasts about 1 month. Radical mastectomy including removal of the axillary lymph nodes was usually performed 4—6 weeks after the termination of the X-ray treatment. Twenty-six of the patients received a complete pre-operative treat-

*) The mammary biopsies were removed by Dr. S. Kaae, usually from the same part of the tumour.

ment, 3 of them (369, 373, 383) a somewhat smaller dose, $150\text{ r} \times 10$ (4 fields), $150\text{ r} \times 7$ (3 fields), and $150\text{ r} \times 12$ (2 fields).

The approximate tumour dose obtained in a mammary tumour by a complete pre-operative treatment was calculated by Dr. S. Kaae to total about 3000—3500 r and about the same tumour dose is considered to be delivered to the surface of an ulcerated mammary tumour.

(3) Classification of the Material.

It is well-known that the appearance as well as degree of differentiation of a mammary carcinoma may differ considerably from one part to another. It may show partly solid neoplastic tissue, partly a pronounced adenomatous structure. Therefore, a histological classification based on the differentiation found in a material of biopsies which only represent a small part of the tumours can only afford a rather uncertain estimate of the degree of differentiation in each individual tumour. Besides, it must be presumed that in several cases biopsy from the untreated tumour fails to give a correct impression of the differentiation predominating in the growth. The same must apply to the biopsies taken prior to and during the radiations, whereas the operation specimens examined in several large sections through the entire tumour give a much better impression of the degree of differentiation. One must therefore be prepared that the biopsy specimen may in some instances differ to some extent from the parent tumour as regards the degree of differentiation. Still, if the radiations constantly or frequently entailed changes in the degree of differentiation, this would undoubtedly be demonstrable in a material of this size, comprising about 150 biopsies.

I have classified the material into solid and adenomatous varieties, but as already mentioned every reservation is made as to the correctness of the grading in each individual case.

In 20 instances the growths were solid carcinomas, of them 2 solid gelatinous carcinomas.

Nine tumours were more or less adenomatous, 6 of them, however, predominantly solid with only a few glandular imitations (not including the cell clusters of a "pseudoacinous" appearance commonly met with in the solid carcinomas). Only one case (No. 379) must be classified histologically as a highly differentiated adenocarcinoma. The estimated degree of differentiation is based on the more or less preserved capacity to form glandular structures, whereas polymorphism and secretion bodies are disregarded, as they are changed by the radiation and the consequent cellular degeneration. The amount of connective tissue in the mammary tumours is not considered to be a fit expression of the degree of differentiation and is therefore not either a factor in the estimation.

(4) Investigations into Alterations in Differentiation Following X-radiation.

In 19 of the solid carcinomas and in 2 (Nos. 379 and 380) of the partly adenomatous growths the differentiation remained completely unchanged in all the specimens.

In a typically solid carcinoma (No. 374) one punch biopsy removed after the administration of $150 \text{ r} \times 5-6$, revealed a few glandular structures. The other 2 punch biopsies as well as the operation specimen, however, showed the same differentiation as the biopsy from the untreated tumour.

In 7 of the partly adenomatous carcinomas the differentiation was found to vary somewhat during and after the radiation. Only in one case (No. 371) a punch biopsy, taken after $150 \text{ r} \times 2-3$ showed a higher differentiation (half solid, half adenomatous) than the original untreated tumour (predominantly solid with a few glandular structures), but the other 5 punch biopsies and the operation specimen showed the same differentiation as the biopsy from the untreated growth. In the remaining 6 cases (Nos. 378, 381, 383, 387, 389, and 394) one or more biopsies taken during the radiations revealed a somewhat lower differentiation than found in the untreated tumours, but the variations were very moderate and most of the specimens revealed the same differentiation (see Figs. 21—22). Therefore, it is evident that the variations in differentiation found in the entire material are few and slight. In my opinion they can be reasonably explained by the uncertainty which must attach to a method which only affords small biopsies from comparatively large tumours with a marked tendency to variations in the differentiation from one part to another.

The differences found in the differentiation proved independent of the X-ray dose and on the whole they appear to be due to chance.

Recurrences were observed in 9 cases, but none of them has been examined microscopically. On an average the patients have only been followed up for 2 years. Consequently the material does not permit of any conclusions as to the relation between the histological appearance of the untreated tumour, the histological radiation reaction, and the end result.

(5) Investigations into Histological Radiation Changes in General in Mammary Carcinoma.

The histological radiation changes encountered in the human mammary carcinomas do not differ fundamentally from those observed previously, and the reader is referred to the description in Chapter IV, Section 4.

Following large doses the tumours were usually characterized by

the formation of ample collagen connective tissue in the stroma, lending the tumours an appearance resembling scirrhus.

Metaplasia to squamous epithelium was not observed.

On the whole the radiation changes in the human mammary growths were less marked than in the other tumours studied, but there are wide variations.

Several cases exhibited a certain disproportion between the degree of the histological radiation reaction and the clinical response to the radiation. For example, Cases 384 and 390 showed a strikingly slight effect of the radiation histologically in spite of satisfactory clinical response, whereas e. g. Cases 374 and 377 showed marked histological radiation changes, but a slight clinical effect. (All four tumours were operable and had received a complete pre-operative X-radiation).

In some cases I observed unaffected or practically unaffected tumour cells during or immediately after the radiation, even in the presence of marked radiation changes in the tumour cells and stroma on the whole. An example is afforded by Case 369 (solid scirrhus carcinoma). During treatment the tumour cells showed pronounced effects of radiation, but 3 days after the treatment was concluded there were islets of practically unaffected neoplastic tissue in a typically fibrous, oedematous connective-tissue stroma showing marked effects of radiation. These cells correspond to *Ewing's* "process of adaptation" (1938), but the radio-resistant cells are *not* more differentiated than the other cells in the tumour. Such findings do not support the repeatedly advanced theory (*Dyroff* (1929), *Warren* (1941 and 1942 1)) that the so-called "persisting cells" which may survive intensive radiation and form the starting point of a recurrence, should be more highly differentiated than the other tumour cells.

Other tumours again were comparatively radio-sensitive. In several instances neoplastic cells were not demonstrable in the operation specimens consisting of several large sections through the tumours which at that time were made up exclusively of fibrous tissue.

As the untreated material only comprises biopsies, never the entire tumour, it was impossible to extend the study to the 4 zones demonstrated in 1941 by *Reuterwall*. The operation specimens showed the regression of the neoplastic tissue and the scirrhus cicatricial healing described by *Reuterwall*, but no distinct zones. Usually the lymph node metastases showed rather less pronounced, but unmistakable radiation changes.

(6) Summary of Chapter VIII.

Chapter VIII treats of histological studies of biopsies removed from 29 patients with mammary carcinoma during and after pre-operative X-radiation. Moreover, operation specimens were available in 20 of the cases. The degree of differentiation, estimated on the basis

of the capacity for glandular imitation, was found to vary somewhat in the biopsies examined during the treatment. This is only natural in view of the relatively small biopsies from large tumours with a marked tendency to show varying differentiation from one part to another. The variations in the differentiation appear to be due to chance and do not exhibit any tendency to increased differentiation following radiation.

The histological changes in the mammary tumours are in all essentials of the same appearance as those found in the tumours already described. The intensity of the radiation changes shows wide variations, often with no relation to the clinical response.

In a certain number of cases one may, even after a complete course of pre-operative X-radiation, encounter almost unaffected neoplastic tissue in a connective-tissue stroma bearing the marks of severe radiation changes. This corresponds to *Ewing's* "process of adaptation", but the radio-resistant, surviving tumour cells were *not* in any case more differentiated than the other tumour cells. Therefore, this does not support the theory that the so-called "persisting cells" which may survive heavy radiation and form the starting point of recurrence, should be more highly differentiated than the other tumour cells.

CHAPTER IX.

Spindle-cell Sarcoma Street

(Transplantation Experiments with Mice).

(1) Material.

Spindle-cell sarcoma Street is a transplantable mouse tumour which was detected in 1940 at the University Institute of Pathological Anatomy, Copenhagen, in the left axilla of a Street mouse injected 4 months previously with oestradiol benzoate into the subcutaneous tissue at another site. Since then the tumour has been kept growing by transplantation into Street mice. The percentage of takes is high, its growth is rapid and it quickly entails death. On the tail the tumour takes in 80—90 per cent., with a latent period of 2—4 weeks and leads to death in the course of a few months, at times metastasizing to the lungs.

In the one hundred odd passages the tumour has retained the same appearance (see Fig. 23), that of a richly cellular undifferentiated spindle-cell sarcoma with slender spindle-shaped cells without fibrils or pigmentation. The nuclei are moderately polymorphous, and there are numerous often slightly abnormal mitotic figures. Frequently there are pyknotic and small necrotic areas. The stroma is very sparse. On the tails the stroma is often more abundant than in the axilla, and the tumours are on the whole more polymorphous, but show fewer mitoses.

As this sarcoma is undifferentiated, it ought to be suitable*) for experiments intended to produce differentiation following X-radiation which should be demonstrable in the form of collagen fibrils as stated by *Dominici & Barcat* in 1907.

(2) Radiation Experiments.

The experiments consisted in X-radiation with the usual technique of the tumours transplanted into the tails of Street mice.

*) One *may*, however, raise the objection against the use of this spindle-cell sarcoma for this purpose that it has never shown any signs of differentiation in the form of collagen fibrils and has perhaps lost this power altogether.

The control material comprises 27 untreated tumours (Nos. 396—422). Nine of them are axillary tumours used for transplantation into the radiated series, whereas 18 are tail tumours derived from the series used for radiation experiments. In addition, 24 axillary tumours from the successive transplantation series (Nos. 423—446) and 4 pulmonary metastases (Nos. 403, 412, 486, and 496) were examined.

The microscopic appearance did not in any respect differ from the 27 actual controls. In none of the tumours was there any sign of differentiation in the form of collagen fibrils.

Experiments:

Single dose of 25 r: (Nos. 447—454, Series 524 and 525 i). A total of 8 mice, killed from 1½ hours to 4 days after the exposure.

Single dose of 300 r: (Nos. 455—464, Series 524 and 525 f). A total of 10 mice, killed from 1½ to 24 hours after the exposure.

Single dose of 500 r: (Nos. 465—469, Series 525 b and Nos. 485—488, Series 504, 505, and 506 b). $5 + 4 = 9$ mice, killed from 1 to 13 days after the exposure.

Single dose of 1000 r: (Nos. 470—474, Series 525 c and Nos. 489—492, Series 504, 505, and 506 c). $5 + 4 = 9$ mice, killed from 1 to 13 days after the exposure.

Single dose of 1500 r: (Nos. 475—479, Series 524 and 525 d and Nos. 493—496, Series 504, 505, and 506 d). $5 + 4 = 9$ mice, killed from 1 to 13 days after the exposure.

Single dose of 2000 r: (Nos. 480—484, Series 524 e and Nos. 497—500, Series 504, 505, and 506 e). $5 + 4 = 9$ mice, killed from 1 to 13 days after the exposure.

Fractionated X-radiation once daily up to a total of 5500 r (200—400 — 500 — 600 — 600 — 600 — 700 — 700 — 400 — 400 — 400 r): (Nos. 501—514, Series 501, 504, 505, and 506 a). A total of 14 mice, killed from 2 days after the second exposure to 3 days after the 11th exposure.

A total of 68 radiated and 51 untreated tumours were examined.

(3) Investigations into Alterations in Differentiation Following X-radiation.

Microscopical examination of the radiated tumours (see Fig. 24) did not in any case reveal signs of increased differentiation in the shape of collagen-fibril formation. On the other hand, there was increased proliferation of the connective-tissue stroma, as expected in analogy with the effect of radiation found in other tumours and in normal connective tissue. This proliferation of young connective tissue, later in the radiation period attended with the formation of collagen fibrils, was, if anything, less marked in this spindle-cell sarcoma than in squamous-cell carcinoma Aka and mammary carcinoma Dlb. How-

ever, it was quite appreciable in several of the growths (see Fig. 25). At no time was I in any doubt that this late production of collagen fibrils, also in the spindle-cell sarcoma, was a reaction on the part of the connective-tissue stroma (in transplanted tumours presumably arising in the cells of the host), in other words that the collagen fibrils did not originate in the sarcoma cells. The latter exhibit the usual radiation changes, i. e. degeneration of the nucleus, swelling and at last disappearance of the protoplasm, so that in the late stages when the collagen fibrils are seen, the cell is in a state of severe degeneration and has in most cases entirely lost its spindle shape.

This tumour did not either show any signs of "orderly differentiation" following radiation.

A few cases, particularly those receiving comparatively small doses, exhibited recurrence of entirely or practically unaffected neoplastic tissue in between the greatly affected tumour cells. Even this neoplastic tissue showed the same low differentiation as the untreated tumours. The "differentiation" in sarcoma following radiation reported by *Dominici & Barcat* (1907 and 1908 1. and 2.) and later by *Clunet* (1910) is in my opinion undoubtedly due to a misinterpretation of the normal proliferation of connective tissue with the formation of collagen fibrils encountered regularly in the late phases of radiation and which at times may rather closely resemble a highly differentiated spindle-cell sarcoma or a fibroma.

(4) Investigations into Histological Radiation Changes in General in Spindle-cell Sarcoma Street.

The histological radiation changes found in the spindle-cell sarcoma are in all essentials of the same nature as those met with in the other tumours, and the reader is referred to Chapter IV, Section 4.

The cellular radiation changes in this tumour are extremely violent, much more marked than in any of the other mouse tumours and on a level with those found in the radium-treated cervical cancers. This is startling in view of the radio-resistance of the tumour which only showed temporary arrest of the growth or regression, but never complete disappearance. These experiments, therefore, afford a *good example of the fact that the clinical response of a malignant tumour to radiation cannot be foretold on the basis of the reaction observed histologically during the treatment.* As mentioned above the connective-tissue proliferation during radiation of this tumour was rather less pronounced than usual, perhaps because of the radio-resistance of the growth. Otherwise it was of the same nature as in the other tumours.

(5) Summary of Chapter IX.

An undifferentiated spindle-cell sarcoma transplanted into Street mice was examined histologically after X-radiation. None of the 68

radiated tumours showed differentiation in the form of collagen fibrils or "orderly differentiation". This is at variance with the studies reported by *Dominici & Barcat* and *Clunet*. The differentiation following radiation described by these authors is in my opinion due to a misinterpretation of the proliferation of connective-tissue with formation of collagen fibrils encountered normally after heavy radiation of a tumour and which at times may rather closely resemble a highly differentiated spindle-cell sarcoma.

The cellular radiation changes in the spindle-cell sarcoma, in all essentials of the same nature as in the other tumours, are extremely marked in spite of the radio-resistance of the tumour and go to show that no conclusions as to the clinical response to the radiation can be based on the histological reactions demonstrable during treatment.

CHAPTER X.

Chondrosarcoma

(Transplantation Experiments with Mice).

(1) Material.

In 1906 *Ehrlich* found a spontaneous chondrosarcoma in a mouse, in the abdomen, adhering to the omentum. This tumour has been kept growing by transplantation and has shown a high percentage of takes (*Ehrlich* 1907). In Street mice the tumour takes in the axilla and thigh in about 30—40 per cent. after a latent period of about 2—4 weeks. My transplantations to the tails, on the other hand, proved unsuccessful. Metastases were not observed in my experiments.

The tumour (Fig. 26) is made up of a filamentous cartilaginous intercellular substance forming a coarse network surrounding islets of about 4—10 cartilage cells with rather large, rounded, slightly to moderately polymorphous nuclei. The chromatin is rather sparse, the nucleolus large, slightly eosinophilic, and there is a moderate number of normal or sometimes mildly pathological mitoses. The protoplasm is pale and ill-defined, showing paler vacuoles in places. Bone formation or calcification has not been observed in untreated tumours. The amount of the cartilaginous intercellular substance varies somewhat, but actual chondroblastic tissue has not been met with. In the common spontaneous necroses in large tumours the nuclei are small, pyknotic or else they are barely discernible as faint shadows and the cartilaginous intercellular substance is more abundant, appearing to be rather swollen and without visible structure. The stroma is sparse, consisting almost exclusively of fine blood-vessels the walls of which are invisible or nearly so. It is only along the edge of the growth that delicate strands of connective tissue are seen between the cartilage cells. Usually the surrounding connective tissue shows marked infiltration of plasma cells and lymphocytes and occasional histiocytes.

The differentiation is rather high, but theoretically an increased differentiation should be demonstrable in the form of increased cartilaginous intercellular substance or bone formation. Calcification in the tumour, on the other hand, is not interpretable as differentiation.

(2) Radiation Experiments.

All the experiments consisted in X-radiation with the usual technique of tumours transplanted into the thighs of Street mice.

The control material comprises a total of 64 tumours (Nos. 515—578). Of them 32 are controls from the radiated series, 10 were used for transplantation into these series, whereas 22 are derived from the successive transplantation series. The great majority were femoral tumours, a few axillary.

Experiments:

Single dose of 25 r: (Nos. 579—589, Series 443 and 444 i). A total of 11 mice, killed from 2 hours to 6 days after the exposure.

Single dose of 300 r: (Nos. 590—602, Series 447 and 448 c). A total of 13 mice, killed from 1½ hours to 5 days after the exposure.

Single dose of 500 r: (Nos. 603—612, Series 431 a). A total of 10 mice killed from 0 hour to 10 days after the exposure.

Single dose of 1000 r: (Nos. 613—622, Series 431 b). A total of 10 mice, killed from 18 hours to 20 days after the exposure.

Single dose of 1500 r: (Nos. 623—630, Series 435, 436, and 437 a). A total of 8 mice, killed from 20 hours to 10 days after the exposure.

Single dose of 2000 r: (Nos. 631—640, Series 442, 443, and 445 b). A total of 10 mice, killed from 10 hours to 17 days after the exposure.

Fractionated X-radiation, 400 r once daily up to total of 6000 r: (Nos. 641—660, Series 442, 443, and 444 a). A total of 20 mice, killed from 0 hour after the 1st to 3 days after the 15th exposure.

A total of 82 radiated and 64 untreated tumours were examined.

(3) Investigations into Alterations in Differentiation Following X-radiation.

Microscopical examination of the radiated tumours showed the cartilaginous intercellular substance to be exactly like that in the untreated tumours. In tumours showing marked effects of radiation the intercellular substance was moderately swollen, structureless, of an appearance like the spontaneous necroses described above (Fig. 27). The increase in the bulk of the cartilaginous intercellular substance corresponds to that observed in the spontaneous necroses and appears to be of the same order of magnitude as the enlargement of the cells induced by the radiation. Consequently, it is not a question of increased formation of cartilaginous intercellular substance. In a few cases showing severe radiation changes there will be calcareous areas, partly in the tumour cells, partly in the intercellular substance. Calcification in a tissue is, however, a common phenomenon of degeneration and cannot be interpreted as a sign of differentiation. Bone formation was not observed.

The appearance of the radiated tumours did not at any time suggest a more "orderly" differentiation.

(4) Investigations into Histological Radiation Changes in General in Chondrosarcoma.

Few reports have been published of histological examination of radiated chondromatous tumours. *Laborde & Cuel* (1922 and 1923), radiating an atypical chondroma, found necrotic areas 15 days later. *Schneider* (1927) after histological examination of a human enchondroma of the hip bone following X-radiation, reported polymorphism, absence of mitotic figures, pyknotoses, vacuolation, giant cells, connective-tissue proliferation, calcification of the cartilage, and vascular sclerosis. No biopsy had been taken prior to radiation, so these findings must be regarded with reservation. *Ewing*, in 1930, described a radiated chondroma of the calcaneus; he found an arrest of tumour growth and calcification in the tumour.

Histological changes observed after X-ray or radium treatment of chondrosarcoma do not, however, appear to have been reported in the literature, and I therefore feel justified in reporting the radiation changes found in this transplanted chondrosarcoma:

The nuclear and protoplasmic changes in the chondrosarcoma following X-radiation do not in any respect differ from those observed in the other tumours. They are very marked, almost as marked as those found in the spindle-cell sarcoma.

The experiment with a single dose of 2000 r may be taken as an example: About 10 hours after the exposure there was complete absence of mitoses, in 2, 3, and 4 days there was moderate enlargement of the cells and nuclei, at times karyorrhexis- and lysis, the nucleoli were enlarged and there were rather few, distinctly abnormal mitoses. The cartilaginous intercellular substance was slightly swollen, but otherwise as it had been prior to radiation. In the course of the subsequent days the enlargement of the nuclei and protoplasm increased, reaching a maximum in about 12 days. At the same time there were violent degenerative nuclear changes and only a few extremely abnormal mitoses (see Fig. 27). The individual islets of cartilage cells now held only from 1 to 3—4 large cells exhibiting monstrous degeneration. The cartilaginous intercellular substance was structureless and moderately swollen. In places there was calcification of cartilage cells and of the cartilaginous intercellular substance. In 17 days the appearance was in part that of violent radiation changes in a necrotic, structureless tissue with chromatin fragments (Fig. 28) and in part that of recurrence of neoplastic tissue showing slight or no effects of radiation.

The reaction of the stroma, on the other hand, differed entirely from all the other tumours examined by me.

There was dilatation of the fine, thin-walled vessels in the tumour and hæmorrhages were seen in the neoplastic tissue. Mild proliferation of the endothelium into the lumen was only observed in a few

instances and thrombi never. Along the edge of the growth, where the spontaneous tumours showed a certain faint tendency to growth of sparse connective tissue, there was a slight increase of vascular, young connective-tissue strands. *Inside the tumour proper on the other hand, there was never any trace of connective-tissue proliferation. In this respect the chondrosarcoma differs strikingly from all the other radiated tumours examined in this material* which usually show proliferation of young connective tissue, later undergoing sclerosis as one of the most characteristic and constant signs of radiation effects. Around the blood-vessels in the chondrosarcoma there were only occasional lymphocytes, plasma cells, histiocytes or leucocytes, but the phagocytic reaction to the radiation was considerably less marked than usual. Like the spindle-cell sarcoma, the tumour is extremely radio-resistant, showing only temporary inhibition or arrest of growth following radiation and no veritable regression. The actual cell death occurring after the radiation is, however, no doubt of a considerably greater extent than indicated by the measurements of the tumours, as the remaining cells show monstrous enlargement. It would seem that the tumour was not capable of (or, perhaps, not in need of) producing proliferation of stroma from the host, requiring only the blood-vessels necessary to supply an adequate amount of blood.

(5) Summary of Chapter X.

Chapter X describes X-radiation experiments with a chondrosarcoma transplanted into mice. A total of 82 tumours were radiated, and the differentiation assessed on the basis of the capacity of the cells to form cartilaginous intercellular substance compared with a control material of 64 tumours. The radiated tumours failed to exhibit an increased formation of cartilaginous intercellular substance or other signs of increased differentiation. A description is given of the histological radiation changes demonstrated in the tumours. The changes in the tumour cells were of the same nature as those described above, whereas connective-tissue proliferation inside the tumour was not met with. In this respect the chondrosarcoma differed essentially from all the other radiated tumours examined by me.

CHAPTER XI

Conclusion.

As mentioned in Chapter II earlier studies of morphological changes in malignant tumours following therapeutic irradiation with X-rays and radium have partly supported *Perthes'* and *Apolant's* theory of degeneration (1903 and 1904), partly been taken to support *Dominici & Barcat's* theory of differentiation (1907) without, however, affording a satisfactory elucidation of this problem.

The histological investigations which I performed with a view to alterations in the differentiation of malignant tumours comprised *squamous-cell carcinomas* (squamous-cell carcinoma Aka (mice), skin carcinoma and cervical carcinoma (human)), *adenocarcinomas* (mammary carcinoma in mice as well as human beings), and *sarcomas* (spindle-cell sarcoma Street and Ehrlich's chondrosarcoma, both in mice). The human tumours were submitted to the customary X-ray and (or) radium irradiation used at the Radium Centre, Copenhagen, whereas the mouse tumours were X-radiated, partly by the fractionated method, imitating the clinical method as closely as possible, partly by single doses varying from 25 to 2000 r. The histological examinations were made a varying length of time after the radiations, in order that they might afford as complete as possible a picture of the changes demonstrable histologically.

The above-mentioned tumours were selected because theoretically they ought to afford the greatest possible chances of exhibiting differentiation, if the latter does occur at all.

Among the entire material, comprising 986 microscopic preparations from a total of 660 tumours I did not in any case find signs of increased, regular "orderly differentiation" in the radiated tumours as one would expect if the radiation produced increased differentiation.

The squamous-cell carcinomas were, in addition, searched for parakeratosis and cornification which constitute easily demonstrable signs of differentiation.

In the *squamous-cell carcinoma in Aka mice* where, theoretically, differentiation should be particularly easy to induce because of the original marked cornification, apparent still in places, the differentiation remained unchanged in all essentials after radiation. A few of the tumours treated with large single doses showed a faint in-

crease in parakeratosis, showing no relation to the period elapsing from radiation, but no definite increase in cornification. The tumours treated with fractionated radiation failed to show any definite increase in parakeratosis or cornification as compared with the controls which at times may exhibit quite marked differentiation, but a faint increase in both cannot be ruled out.

In my opinion these faint and rare changes cannot be attributed with any importance as factors in tumour destruction. I do not either interpret them as a sign of differentiation, but consider the most probable explanation to be a selection of the comparatively few fairly differentiated and fairly radio-resistant tumour cells, the undifferentiated, more radio-sensitive cells degenerating and perishing more readily. Theoretically, I had expected to find a far greater relative increase in the number of highly differentiated tumour cells than I actually did.

On the other hand, one cannot quite rule out the possibility that the undifferentiated, radio-sensitive cells are killed by the radiation, whereas the more differentiated cells persist and go on differentiating like the cells of normal skin, a fact which is demonstrated by the experiments with normal mouse skin.

If this explanation were tenable in the case of tumours also, one would, however, have to expect the differentiation to be more diffuse and more marked, but it only involved a few, small areas in quite few tumours.

Human skin carcinoma did not exhibit signs of differentiation following radiation, not even a relative increase in the number of the fairly differentiated cells.

Cervical carcinoma did not show signs of increased differentiation and only 1 case (No. 234) revealed a marked increase in parakeratosis. In this instance there was a pre-existing excessive parakeratosis, and the phenomenon is presumably relative, due to slower destruction and absorption of the differentiated cells compared with the radio-sensitive undifferentiated cells.

Adenocarcinoma, mouse as well as human mammary carcinoma, failed to show any signs of increased differentiation in the form of increased glandular imitation.

Neither did the radiated *mouse sarcomas* exhibit any signs of relative or absolute increase in differentiation, the criteria being the tendency to form collagen fibrils in *the spindle-cell sarcoma* and the tendency to form cartilaginous intercellular substance in *the chondrosarcoma*.

On the whole, the experiments show that increased differentiation was not demonstrable histologically in any case and that only a few exhibited a faint relative increase in the number of the more differentiated tumour cells which is not surprising as the more differentiated tumour cells are more radio-resistant. In other words, the experiments

speak definitely against *Dominici & Barcat's* theory of differentiation, as studies of this extent with so varied a material and so varied a dosage of radiation must be expected to elicit differentiation, if it does occur at all.

There are numerous sources of error to account for the reports of differentiation following radiation published by so many workers, among them also experienced tumour pathologists.

The observation most commonly reported in the literature is increased parakeratosis and cornification which may easily be confused with tumour cells showing marked effects of radiation, a degenerated nucleus and an eosinophilic protoplasm.

Tumours covered by normal skin often, and particularly after radiation, show tongue-shaped masses extending down into the dermis in between the tumour cells. When the tumour as well as the epithelium show marked effects of radiation, it may be extremely difficult to decide whether a degenerated horny pearl surrounded by granulation tissue belongs to the carcinoma or to the non-carcinomatous epidermis.

As to adenocarcinoma, errors are apt to be made in the interpretation of the frequent "pseudoacinous cavities".

In my opinion the differentiation stated to take place in sarcoma in the form of collagen fibrils is no doubt due to misinterpretation of the normal proliferation of fibroblasts which is a characteristic feature in radiated tumours and which later leads to the formation of collagen fibrils.

Moreover, chance plays a marked rôle. Most of the earlier investigations into this subject have not paid sufficient regard to the control material. One biopsy taken before the institution of treatment is of limited value, and in my opinion studies of human tumours are only applicable when the materials are large, whereas it is not warrantable to draw conclusions from biopsies derived from one single case. My material has in numerous instances shown that some tumours appear to exhibit a higher, others a lower differentiation following radiation, whereas an evaluation of the material as a whole reveals unaltered differentiation. This uncertainty applies particularly to mammary tumours in which the differentiation may show wide variations from one part to another in the same growth.

In studies of this particular nature the value of spontaneous tumours is by far exceeded by that of transplanted tumours as they are derived from the same tumour and as their histological architecture can be compared with numerous controls in every experiment. In such cases the variations from one transplanted tumour to another are no greater than may sometimes be found within the same spontaneous tumour, less if anything, as tumours transplanted through several passages have a certain tendency to assume a more uniform histological appearance.

Furthermore, in transplanted tumours, the same type of tumour may be exposed to radiations with widely different dosage.

Lastly, the interpretation of the histological changes is of the utmost importance. Several workers, *inter alia* Glücksmann, Spear and Donaldson interpret enlargement of the cells following radiation as a sign of differentiation. If that is so, differentiation takes place following any heavy irradiation of cells, as cellular enlargement is a constant finding after irradiation. It is not, however, in accordance with the generally accepted criteria of differentiation to interpret a ballooned monstrous cell with a partially destroyed nucleus as a sign of differentiation, whereas the cellular changes correspond exactly to severe cell degeneration. It is true that at a certain stage of the cornification of epithelium, the cells grow larger and flatter, forming intercellular bridges, and that the nuclei degenerate, but this takes place in a regular "orderly" way, showing increasing differentiation. Radiated tumour cells, on the other hand, are disposed in a disorderly fashion, scattered, of varying size, having an eosinophilic protoplasm and showing signs of marked nuclear enlargement and degeneration. Intercellular bridges are never observed after irradiation.

Unlike cellular enlargement, parakeratosis and cornification are unmistakable signs of differentiation, and unlike Glücksmann I consider them the most valuable criteria by which the degree of differentiation in radiated squamous-cell carcinoma can be judged.

One can raise the objection against my experiments that they deal exclusively with morphological changes which do not allow of any conclusions as to the function of the cells. This objection cannot be dismissed without comments, as a histological preparation from a tumour is merely an instantaneous photograph of numerous changing processes and moreover, being fixed and stained, an artificial product, and as microscopic examination only shows certain rough changes in the tumour cells. This applies, however, to all morphological investigations and therefore also to the histological aspect of the concept known as differentiation.

Paying due regard to these objections *I must conclude from my investigations that therapeutic irradiation of a number of theoretically suitable tumours with X-rays or radium is not followed by histologically demonstrable changes which can be interpreted as criteria of differentiation with our present knowledge of morphology.*

In my opinion the result of my investigations must be that *the dosage in clinical radiotherapy should not be adapted to alterations, if any, in the differentiation during or after the radiation, as has been advocated by Friedman (1939—1940).* Unlike Glücksmann and associates I am of the opinion, based on my studies, *that the histologically demonstrable reaction in a malignant tumour during radiation is inapplicable as a means of foretelling the clinical response of the tumour to the radiation.* In fairly radio-resistant tumours I have often ob-

served cancer cells showing marked effects of the radiation (for example in certain human mammary tumours and in the spindle-cell sarcoma and chondrosarcoma in mice), and reversely I have at times found a surprisingly slight histological reaction in tumours which proved to show satisfactory clinical response to radiation (for example several human basal-cell skin carcinomas and human mammary carcinomas). As far as I can see, *the histological reaction in a malignant tumour during radiation is not a fit standard by which one can adapt the radiation dose.*

This can be done much more safely on the basis of clinical observation of the patient and tumour during radiation.

I am not inclined to think that the histological reaction in a tumour during radiation is a suitable prognostic sign. My material is not, however, applicable in the study of this question, its aim being to investigate the differentiation in malignant tumours on the basis of histological reactions induced by radiation. Therefore, the mouse tumours were studied during or at most a few weeks after the radiation, before there was a possibility of recurrences, and the human material was not followed up for a sufficient length of time after the radiation to suggest anything as to the ultimate prognosis. For this end the human material is also too small and too inhomogenous.

The histological changes observed in the radiated tumours are described in a separate section at the end of each chapter. *They are in all essentials the same in the various types of tumours, corresponding entirely to the criteria which are generally accepted as signs of cellular degeneration and thus confirm Perthes' and Apolant's theory of degeneration.* The cellular changes may frequently be present in a mild degree in areas of spontaneous degeneration in untreated control preparations from the same tumours. To me there is no doubt *that the histologically demonstrable changes in the radiated tumours are signs of degeneration, interpretable as being of non-specific nature, as its individual elements are frequently found, in a milder degree it is true, in the untreated tumours.*

Summary.

Introduction. During the half century that X-rays and radium have been used in the treatment of cancer the question has been raised repeatedly as to whether these rays possess a power to induce increased differentiation in malignant tumours and whether their curative effect might be partly or wholly due to increased differentiation. As earlier investigations have been sporadic and their results conflicting, the present work was undertaken in an endeavour to elucidate the problem by means of a large material.

Chapter I. In this paper differentiation is taken to mean transformation from an unripe general condition to a more ripe, specialized state.

The principles governing the interpretation of the differentiation observed in malignant tumours are discussed on the basis of the literature available and it is emphasized that the degree of differentiation is merely one of the properties — an extremely important one it is true — deciding a tumour's biological malignancy which in turn is only one of the numerous factors determining a tumour's clinical malignancy.

Chapter II reviews earlier investigations into morphological cellular changes in malignant tumours following therapeutic irradiation with X-rays and radium from the point of view of differentiation. First a few radiobiological investigations are dealt with and it is emphasized that the effects of X-rays and radium are considered to be identical in all essentials.

Hitherto the histological studies have afforded conflicting results, one group of workers supporting the view advanced by *Perthes* (1903) and *Apolant* (1904) that histologically demonstrable radiation changes in cancer cells should be a sign of degeneration, while another group of workers incline to the theory suggested in 1907 by *Dominici & Barcat* that the main effect of the radiation should be due to increased differentiation (ripening) of the tumour cells. Most of the studies are sporadic and have not been planned with a special view to phenomena of differentiation. Recently *Glücksman* and associates, in major papers, have, however, maintained that radiation induces differentiation in malignant tumours. They consider this differentiation a valuable

prognostic sign which should be observed in the course of radiotherapy.

The last section of the chapter describes the planning of my investigations for which a selection was made of a number of characteristic mouse and human tumours, which theoretically ought to be capable of displaying easily demonstrable differentiation after radiation.

Chapter III describes the technique used in the transplantations, measurements of the tumours, preparation of removed tumour tissue and X-radiation of the mouse tumours. The tumour dose in the radiated mouse tumours is estimated as being equal to or slightly higher than the dose measured in air.

Chapter IV reports X-radiation experiments with squamous-cell carcinoma Aka, a transplantable mouse carcinoma, originally distinctly cornifying, but which has become undifferentiated with sporadic signs of parakeratosis and cornification in the later passages. In the main the differentiation remained completely unchanged, following radiation with single doses as well as by the fractionated method. A few of the tumours receiving large single doses, however, showed a quite slight increase in parakeratosis, independent of the length of time elapsing from the radiation, but no definite increase in cornification. In fractionated radiation there was no definite increase in parakeratosis or cornification as compared with the controls which at times may display marked differentiation, although a very slight, inconstant increase in both cannot be ruled out. Radiation was not in any case followed by signs of increased "orderly differentiation". The extremely slight, inconstant increase in parakeratosis and doubtful increase in cornification are considered to play no part in the process of destruction and are not signs of radiation-induced differentiation. The most probable explanation is that a selection takes place of the comparatively few fairly differentiated tumour cells, whereas the undifferentiated, more radio-sensitive cells are more apt to degenerate and be absorbed after the radiation. Mention is made of another and less probable explanation, viz. that the undifferentiated, radio-sensitive tumour cells perish after the radiation, while the more differentiated, radio-resistant tumour cells persist and go on differentiating. Experiments with normal mouse skin following fractionated X-radiation show that the latter explanation obtains in the case of normal mouse skin. The histologically demonstrable changes in the 108 X-radiated tumours are described; they are all interpreted as signs of cell degeneration.

Chapter V. Twenty-two human skin carcinomas, basal-cell as well as squamous-cell, were examined after therapeutic irradiation with X-rays or radium. The differentiation remained unchanged after the

radiations and a relative increase was not found either in parakeratosis or cornification. A description is given of the histological radiation changes observed in the tumours, some of which showed a limited radiation reaction in spite of satisfactory clinical effect.

Chapter VI describes the histological changes in 31 human carcinomas of the cervix uteri following therapeutic irradiation with X-rays and radium. More than half the tumours were undifferentiated or rather undifferentiated squamous-cell carcinomas, the remainder rather highly differentiated squamous-cell growths, and one cornifying. The amount of parakeratosis as well as other signs of differentiation remained unchanged after the radiations except for negligible variations. Only one case of an excessively parakeratotic carcinoma showed a relative increase in parakeratosis. The increase in parakeratosis is considered to be due to a selection of the more differentiated parakeratotic cells, whereas the non-parakeratotic cells undergo a more rapid degeneration and are absorbed.

Chapter VII. An undifferentiated solid mammary carcinoma, transplanted into mice, was used for X-radiation experiments. The differentiation remained unchanged in all 77 radiated tumours, in spite of the fact that a few untreated metastases showed an undoubted capacity for differentiation in the form of atypical glandular imitations.

Chapter VIII describes histological examinations of biopsies taken from 29 patients with mammary carcinoma following pre-operative X-radiation. Moreover, operation specimens were examined after mastectomy in 20 of the cases. The degree of differentiation, expressed as the capacity for glandular imitation, was somewhat varied as is only natural in view of the relatively small biopsies from large tumours displaying a marked tendency to variation in the differentiation from one part to another. These variations appeared to be due to chance, and there was no tendency to increased differentiation after radiation. Wide variations were found in the intensity of the histological radiation changes, often bearing no relation to the clinical response of the growth.

Chapter IX. An undifferentiated spindle-cell sarcoma, transplanted into mice, was examined histologically after X-radiation. Not one of the 68 radiated tumours exhibited differentiation in the form of collagen fibrils or "orderly differentiation". In spite of the marked radio-resistance of this growth, the radiation changes were violent.

Chapter X deals with X-radiation experiments with a chondrosarcoma, transplanted into mice. The differentiation was judged by the capacity to form cartilaginous intercellular substance in the 82 ra-

diated tumours as compared with the controls. Increased formation of cartilaginous intercellular substance or other signs of "orderly differentiation" were not observed. A description is given of the histological radiation changes in the tumours. In all essentials the changes in the tumour cells were identical with those encountered in the other tumours studied, but the reaction of the stroma differed essentially from the latter, connective-tissue proliferation in the tumour proper not being observed in any case.

Chapter XI. Among the entire material, comprising 986 microscopic preparations from a total of 660 tumours, I did not in any case find signs of increased regular and "orderly differentiation" in the radiated tumours. In squamous-cell carcinoma in Aka mice which theoretically ought to be particularly suitable for experiments intended to induce differentiation, the latter remained unchanged in all essentials after the radiations. A few cases revealed a very slight increase in parakeratosis and a doubtful increase in cornification. Marked increase in parakeratosis was observed in one of the cervical carcinomas in which excessive parakeratosis had been observed prior to radiation. None of the other tumours displayed any signs of increased differentiation. The increase in parakeratosis and doubtful increase in cornification found in a few cases is considered to have been due to a relative increase in the more differentiated, radio-resistant tumour cells, whereas the undifferentiated, more radio-sensitive tumour cells undergo a more rapid degeneration and are absorbed after the radiations.

In conclusion it is stated that therapeutic irradiation of a number of theoretically suitable tumours with X-rays or radium was not followed by histologically demonstrable changes interpretable, with our present knowledge of morphology, as criteria of differentiation.

In my opinion the result of these investigations must be that *the dosage used in clinical radiotherapy should not be adapted to alterations, if any, in the differentiation during or after the radiations.*

In the present material *the histologically demonstrable reaction in the tumours proved inapplicable as a means of foretelling the clinical response of the tumours to the radiation, and it is therefore not considered a fit standard by which one can adapt the dose in radiotherapy.* The material does not allow of definite conclusions as to the relation between the histological radiation reaction and the ultimate prognosis.

The histological changes observed in the radiated tumours are in all essentials the same in the various types of tumours, corresponding exactly to a cellular degeneration interpreted as being of non-specific nature, as its individual elements are frequently found, in a milder degree it is true, in the untreated tumours.

Resumé.

Indledning: I det halve aarhundrede, røntgen- og radiumstraalerne har været anvendt i behandlingen af kræft, har det spørgsmaal ofte været rejst, om straaerne har evne til at fremkalde øget differentiering i maligne tumorer, og om bestraaelingernes helbredende virkning helt eller delvis skyldes øget differentiering. Da de hidtidige undersøgelser herover har været spredte og resultaterne modstridende, er det hensigten i dette arbejde at tage problemet op til bearbejdelse paa bred basis.

Kapitel I. Ved differentiering forstaas overalt i dette arbejde en omdannelse fra en mere umoden generel tilstand til en mere moden specialiseret.

Principperne for bedømmelsen af en malign tumors differentiering gennemgaas paa basis af den foreliggende litteratur, og det betones, at en tumors differentieringsgrad kun er een af de egenskaber — omend meget vigtig —, der betinger en tumors biologiske malignitet, som igen kun er en af de mange faktorer, der afgør en tumors kliniske malignitet.

Kapitel II. De tidligere undersøgelser over morfologiske celleforandringer i maligne tumorer efter therapeutiske røntgen- og radiumbestraalinger gennemgaas med henblik paa differentiering. Der omtales først nogle straalebiologiske undersøgelser, og det fremhæves, at røntgen- og radiumbestraaling principielt anses for at virke ens.

De hidtidige histologiske undersøgelser har givet modstridende resultater, idet een gruppe undersøgere deler den af *Perthes* (1903) og *Apolant* (1904) fremsatte anskuelse, at de histologisk iagttagelige straalereaktioner i cancerceller er udtryk for en degeneration, medens en anden gruppe undersøgere støtter den af *Dominici & Barcat* i 1907 fremsatte teori, at straalernes hovedvirkning beror paa en øget differentiering (modning) af tumorcellerne. De fleste undersøgelser er spredte og er ikke tilrettelagt med specielt henblik paa differentieringsfænomener. I store arbejder fra de senere aar af *Glücksmann* og medarbejdere har disse imidlertid ment at vise, at straaerne frem-

kalder en differentiering i maligne tumorer, og at denne differentiering er et værdifuldt prognostisk tegn, som bør følges under straalet-behandlingen.

Egne histologiske undersøgelser planlægges, idet en række karakteristiske tumortyper, dels fra mus og dels fra mennesker, som teoretisk skulde være velegnede til fremkaldelse af let iagttagelig differentiering efter bestråling, udvælges.

Kapitel III. Teknikken beskrives ved transplantationer, tumormaalinger, præparation af udtaget tumurvæv og røntgenbestraalinger af musetumorerne. Dosis i de bestraaledede musetumorer skønnes at være lig eller lidt højere end dosis i luft.

Kapitel IV. Der beskrives røntgenbestraalingsforsøg med carcinoma planocellulare Aka, et transplantabelt musecarcinom, der oprindeligt har været udtalt forhornende, men i de senere passager har været udifferentieret med sporadisk parakeratose og forhorning. Differentieringen er i det væsentlige ganske uændret efter saavel enkeltbestraalinger som fraktioneret bestråling; i nogle af de med større enkelt-doser behandlede tumorer ses dog en ganske let forøgelse af parakeratose uafhængig af undersøgelsestidspunktet, men ingen sikker forhorningsforøgelse. I de fraktionerede bestrålingsforsøg ses ingen sikker forøgelse af parakeratose eller forhorning ved sammenligning med kontrollerne, der af og til kan vise betydelig differentiering, omend en ganske let inkonstant forøgelse af begge dele ikke kan udelukkes. Der er i intet tilfælde fundet tegn paa øget »ordnet differentiering« efter bestråling. Den ganske ringe inkonstante forøgelse af parakeratose og tvivlsomme forhorningsforøgelse anses for betydningsløs ved tumordestruktionen og er ikke udtryk for en differentiering som følge af bestrålingen. Den mest sandsynlige forklaring er en udvælgelse af de relativt faa mere differentierede tumorceller, medens de udifferentierede mere straalefølsomme celler i højere grad degenererer efter bestrålingen og resorberes. Man omtaler en anden og mindre sandsynlig forklaring, at de udifferentierede straalefølsomme tumorceller dør efter bestrålingen, medens de mere differentierede straalesistente tumorceller bevares og fortsætter deres differentiering. Det vises ved forsøg med normal musehud efter fraktioneret røntgenbestråling, at denne forklaring maa antages for den normale musehuds vedkommende. De histologisk iagttagelige forandringer i de 108 røntgenbestraalede tumorer beskrives; de er alle udtryk for celledeneration.

Kapitel V. 22 humane hudcarcinomer dels baso- dels planocellulære er undersøgt efter therapeutiske røntgen- eller radiumbestraalinger. Differentieringen er uændret efter bestrålingerne, og der er ikke fundet relativ forøgelse af parakeratose eller forhorning. De histo-

logiske straaaleforandringer i tumorerne beskrives, intensiteten af straalereaktionen er i flere af tumorerne ret ringe trods god klinisk effekt.

Kapitel VI. I dette kapitel beskrives de histologiske forandringer i 31 humane carcinomer paa portio uteri efter therapeutiske røntgen- og radiumbestraalinger. Godt halvdelen af materialet er udifferentierede eller ret udifferentierede planocellulære carcinomer, resten ret højt differentierede planocellulære tumorer, en enkelt forhornende. Parakeratosemængde og differentiering iøvrigt er uændret efter bestraalingerne fraset ubetydelige svingninger, og der paaavises kun relativ forøgelse af parakeratose i et enkelt i forvejen excessivt parakeratotisk carcinom. Parakeratoseforøgelsen menes at skyldes en udvælgelse af de mere differentierede parakeratotiske celler, medens de ikke-parakeratotiske celler hurtigere degenererer og resorberes.

Kapitel VII. Et udifferentieret solidt mammacarcinom transplanteret paa mus er anvendt til røntgenbestraalingsforsøg. Differentieringen findes uændret i de 77 bestraalede tumorer, skønt tumor i enkelte ubehandlede metastaser har vist utvivlsom evne til differentiering i form af atypiske kirtelimitationer.

Kapitel VIII. Der beskrives histologiske undersøgelser af biopsier fra 29 patienter med carcinoma mammae efter præoperativ røntgenbestraaling; i 20 af tilfældene foreligger tillige undersøgelser af operationspræparater efter amputation. Differentieringsgraden maalt ved den kirtelimiterende evne er noget svingende, som man maa vente, da det drejer sig om relativt smaa biopsier fra store tumorer med en betydelig tendens til varierende differentieringsgrad fra omraade til omraade. Differentieringsforskellene har et tilfældigt præg og viser ingen tendens til øget differentiering efter bestraaling. De histologiske straaaleforandringer i tumorerne varierer betydeligt i intensitet, ofte uafhængigt af tumors kliniske reaktion.

Kapitel IX. Et udifferentieret tencellessarcom transplanteret paa mus er undersøgt histologisk efter røntgenbestraalinger. I de 68 bestraalede tumorer er i intet tilfælde fundet differentiering i form af dannelse af collagene fibriller eller »ordnet differentiering«. De iagttagne straaaleforandringer er meget voldsomme, skønt tumor er meget straaleresistent.

Kapitel X. Der beskrives røntgenbestraalingsforsøg med et paa mus transplantabelt chondrosarcom. Differentieringen er bedømt ved evnen til bruskgrundsubstansdannelse i de 82 bestraalede tumorer sammenlignet med kontrolmaterialet. Der iagttages ikke øget dannelse af bruskgrundsubstans eller tegn paa »ordnet differentiering«. De histo-

logiske straalearandringer i tumorerne beskrives; medens tumorcel-
lernes forandringer principielt er af samme natur som i de øvrige
undersøgte tumorer, adskiller stromareaktionen i denne tumor sig af-
gørende fra de øvrige, idet der aldrig ses bindevævsproliferation inde
i selve tumor.

Kapitel XI. Ved bedømmelse af hele det histologiske materiale, der
omfatter 986 mikroskopier fra 660 tumorer, har jeg intetsteds fundet
tegn paa øget regelmæssig og »ordnet differentiering« i de bestraaledede
tumorer. I det planocellulære carcinom paa Aka mus, som teoretisk
skulde være særlig velegnet til fremkaldelse af differentiering, er
denne i det væsentlige ganske uændret efter bestraalingerne. I nogle
faa tilfælde er set en ganske ringe parakeratoseforøgelse og en tvivl-
som øget forhorning. I een af tumorerne fra portio uteri er fundet en
betydelig parakeratoseforøgelse i et i forvejen excessivt parakerato-
tisk carcinom. Ingen af de øvrige tumorer har vist tegn paa øget
differentiering. De faa tilfælde af parakeratoseforøgelse og tvivlsom
øget forhorning menes at skyldes en relativ forøgelse af de mere dif-
ferentierede og straaleresistente tumorceller, idet de udifferentierede
og mere straalefølsomme tumorceller hurtigere degenererer efter be-
straalingerne og resorberes.

*Det konkluderes, at der efter therapeutiske røntgen- eller radium-
bestraalinger af en række teoretisk velegnede tumorer ikke er fundet
histologisk iagttagelige forandringer, som med vor nuværende viden
om morfologi kan opfattes som kriterier paa en differentiering.*

Konsekvensen af disse undersøgelser maa efter min mening blive,
at man ikke i den kliniske straaletterapi bør tilrettelægge doseringen
med henblik paa mulige differentieringsændringer under eller efter
bestraalingerne.

I det foreliggende materiale har den histologisk iagttagelige reak-
tion i tumorerne ikke givet brugbare oplysninger om, hvorledes tumo-
rerne vilde reagere under bestraalingen, og det anses derfor ikke for
hensigtsmæssigt at benytte den histologiske reaktion i en malign tu-
mor under bestraalingen til fastlæggelse af straaledosis. Materialet
tillader ikke sikre slutninger angaaende relationen mellem den histo-
logiske straalereaktion og den endelige prognose.

*De iagttagne histologiske forandringer i de bestraaledede tumorer er
principielt ens for de forskellige tumortyper, og svarer ganske til en
celledeneration, der opfattes som uspecifik, da dens enkelte elemen-
ter ofte genfindes i lettere form i de ubehandlede tumorer.*

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(Abbreviations in conformity with the Quarterly Cumulative Index Medicus. The figures in brackets indicate the pages in the present work on which the respective author has been cited.)

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All the figures are photomicrographs, enlarged $\times 300$.

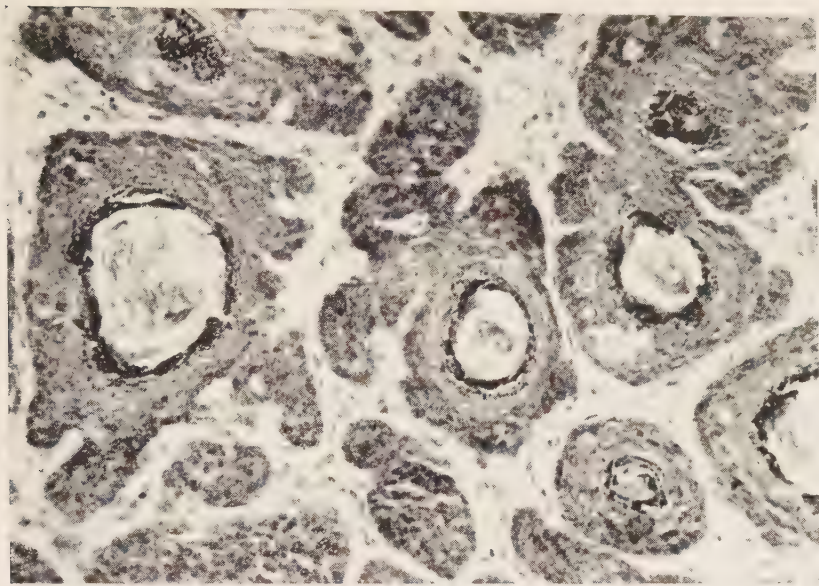


Fig. 1.

Squamous-cell carcinoma Aka. Spontaneous tumour. A highly differentiated cornifying squamous-cell carcinoma with numerous keratohyaline granules and large horny pearls. (After *Engelbreth-Holm*, 1944).

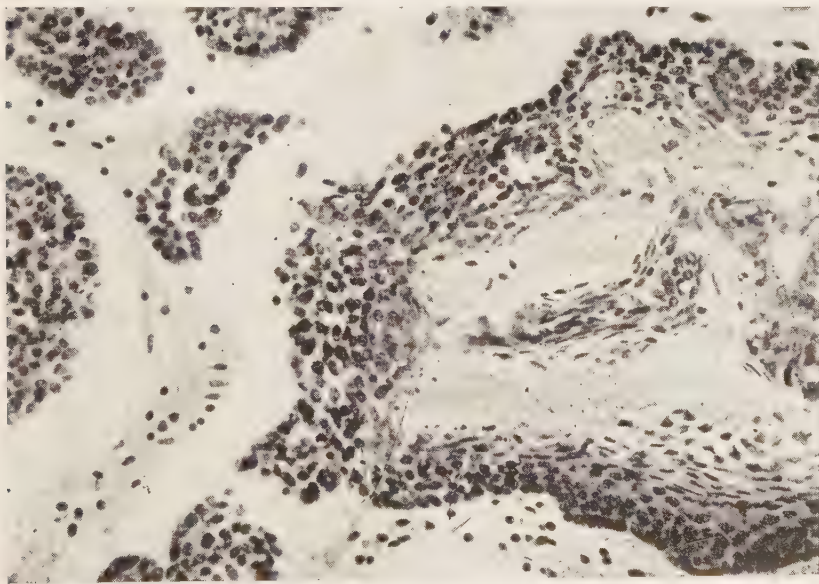


Fig. 2.

Squamous-cell carcinoma Aka, 2nd passage, still with marked cornification, but less than the spontaneous tumour, and only a few keratohyaline granules. (After *Engelbreth-Holm*, 1944).

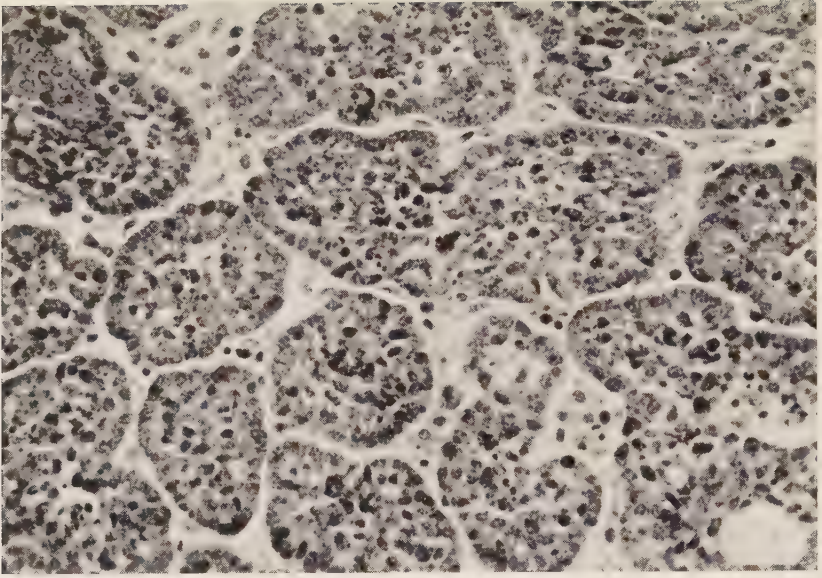


Fig. 3.

Squamous-cell carcinoma Aka, 4th passage. Undifferentiated squamous-cell carcinoma showing numerous mitotic figures and moderate polymorphism. No parakeratosis or cornification. (After *Engelbreth-Holm*, 1944).

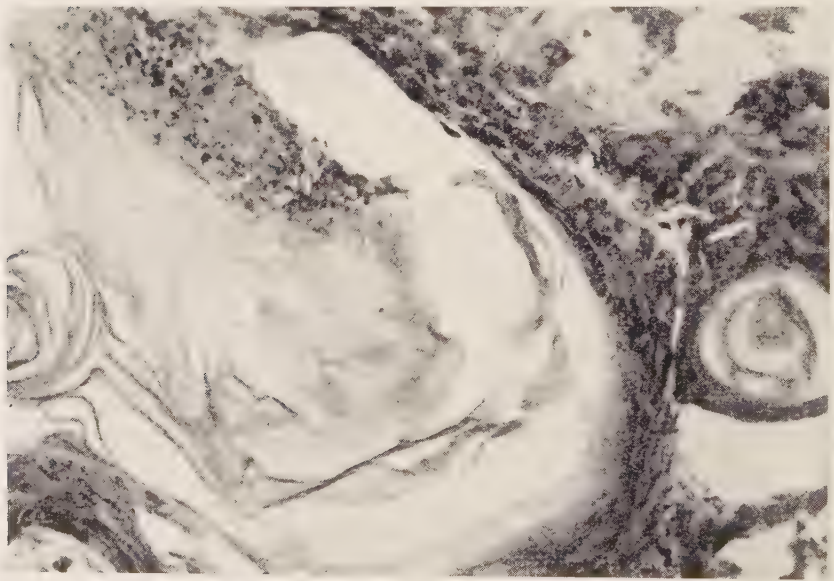


Fig. 4.

Squamous-cell carcinoma Aka (No. 83), Series 144, untreated control. Marked cornification with large and small horny pearls.

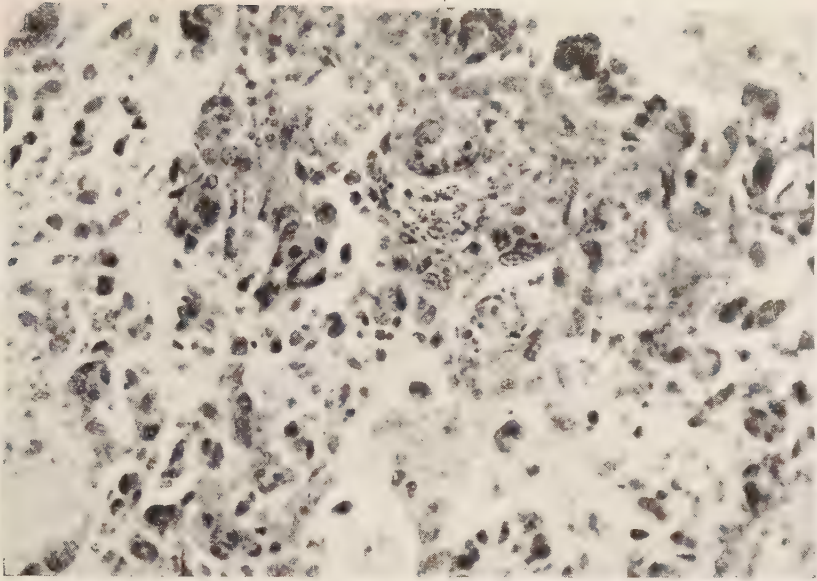


Fig. 5.

Squamous-cell carcinoma Aka (No. 123), Series 159 d. Killed 6 days after 2000 r. Squamous-cell carcinoma showing mild parakeratosis in the centre of the picture (γ). Marked effects of radiation (enlarged, polymorphous, hyperchromatic nuclei, at times multiple).

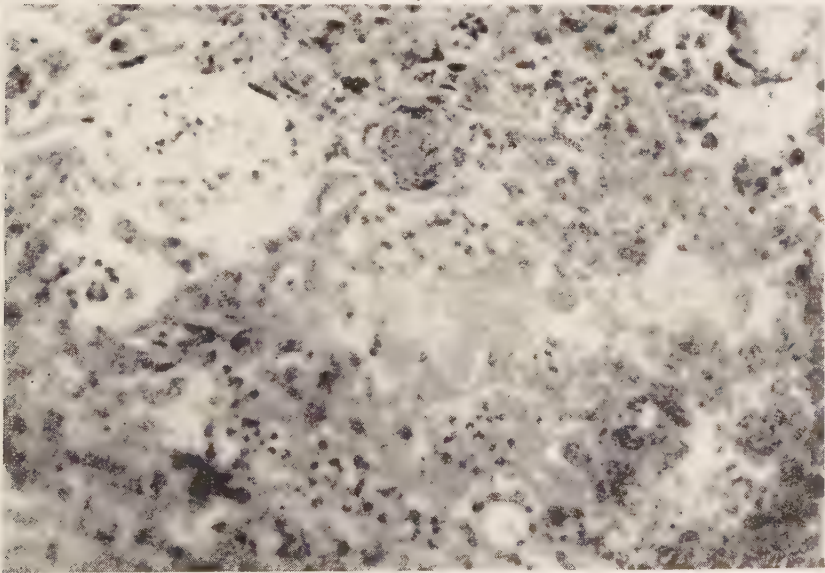


Fig. 6.

Squamous-cell carcinoma Aka (No. 165), Series 163 a. Killed after $300 \text{ r} \times 11 = 3300 \text{ r}$. Squamous-cell carcinoma showing marked effects of radiation, the protoplasmic degeneration resembling parakeratosis. No parakeratosis or cornification in this section.

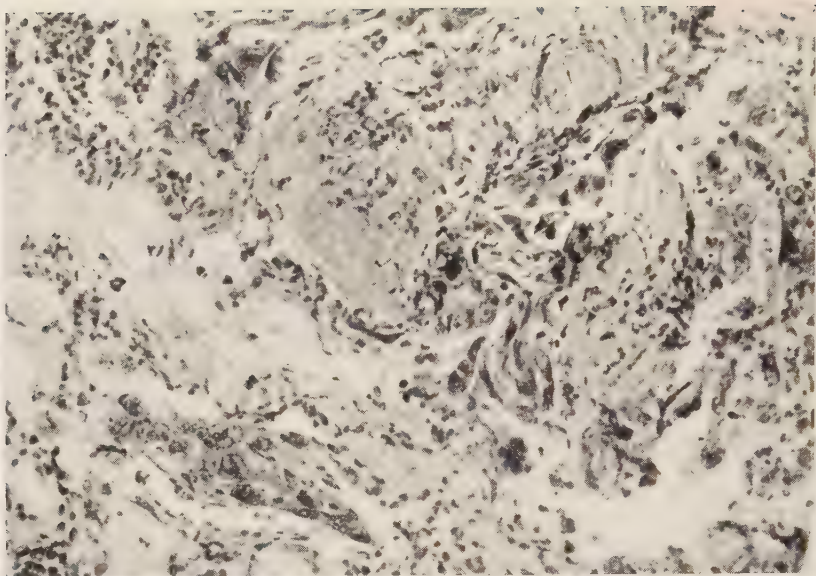


Fig. 7.

Squamous-cell carcinoma Aka (No. 167), Series 163 a. Killed after $300\text{ r} \times 15 = 4500\text{ r}$. Marked effects of radiation in a tumour partly undifferentiated, partly showing pronounced parakeratosis and cornification.

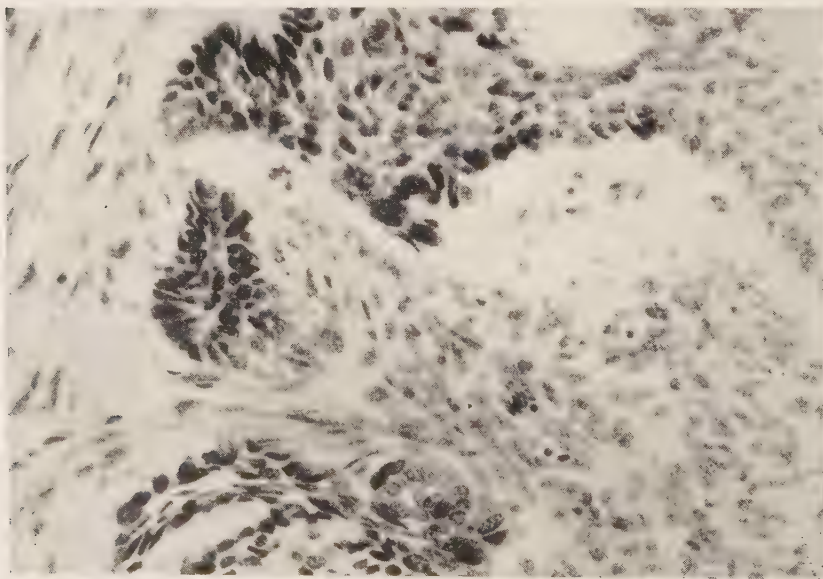


Fig. 8.

Skin carcinoma (No. 197). Excision biopsy after $150\text{ r} \times 1$: Basal-cell carcinoma (*Krompecher* type). A typical basal-cell growth showing islets and strands of slightly polymorphous cells. No differentiation or signs of radiation changes.

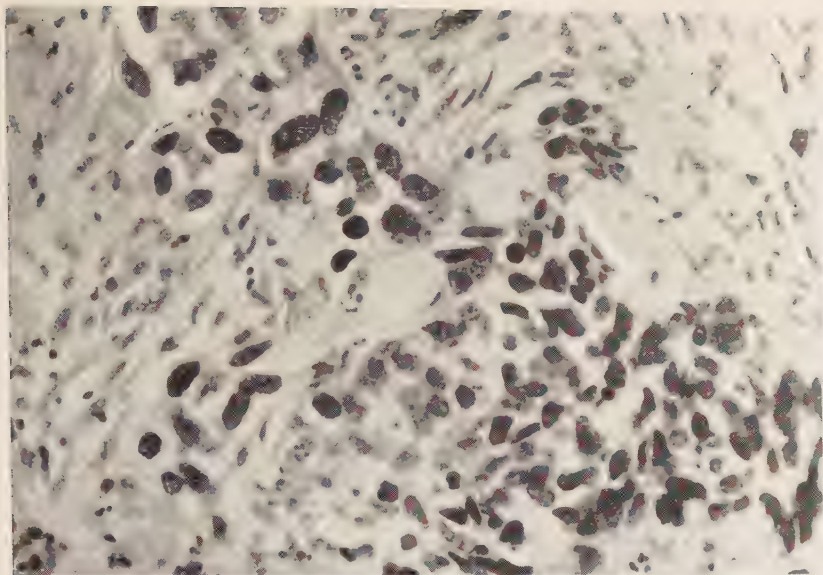


Fig. 9.

Same growth as in Fig. 8. Excision biopsy after 3075 r. Basal-cell carcinoma without differentiation, showing marked effects of the radiation in the form of enlarged, polymorphous, hyperchromatic nuclei in a granulation tissue.

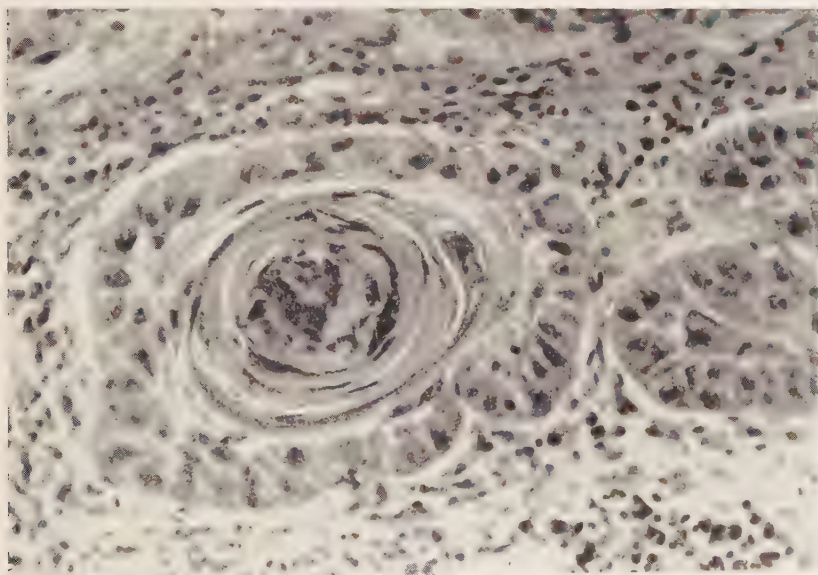


Fig. 10.

Skin carcinoma (No. 214). Excision biopsy from the untreated tumour: Parakeratotic squamous-cell carcinoma. A rather highly differentiated squamous-cell carcinoma with a parakeratotic pearl.

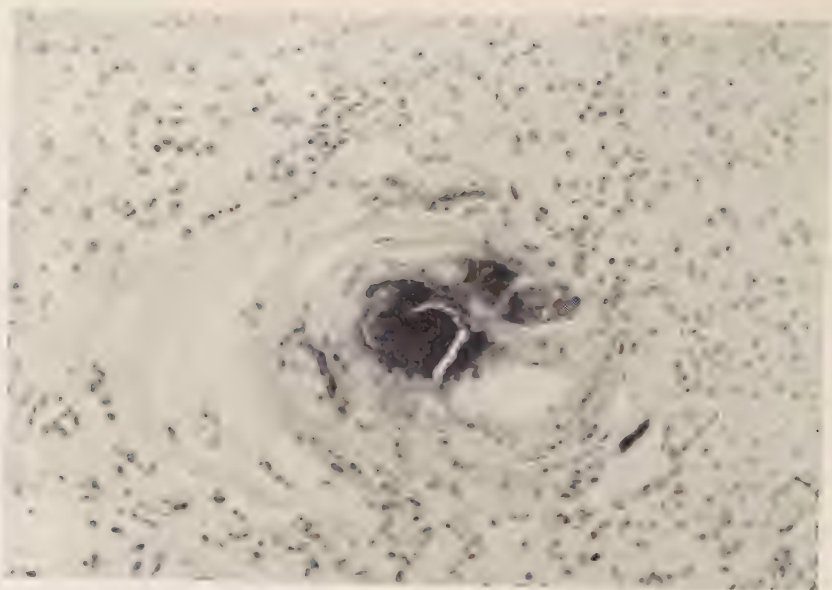


Fig. 11.

The same growth as in Fig. 10. Excision biopsy after 6400 r. A calcified parakeratotic pearl in a granulation tissue with numerous leukocytes. Only the central, parakeratotic, calcareous area is preserved, the peripheral, basal layers destroyed.

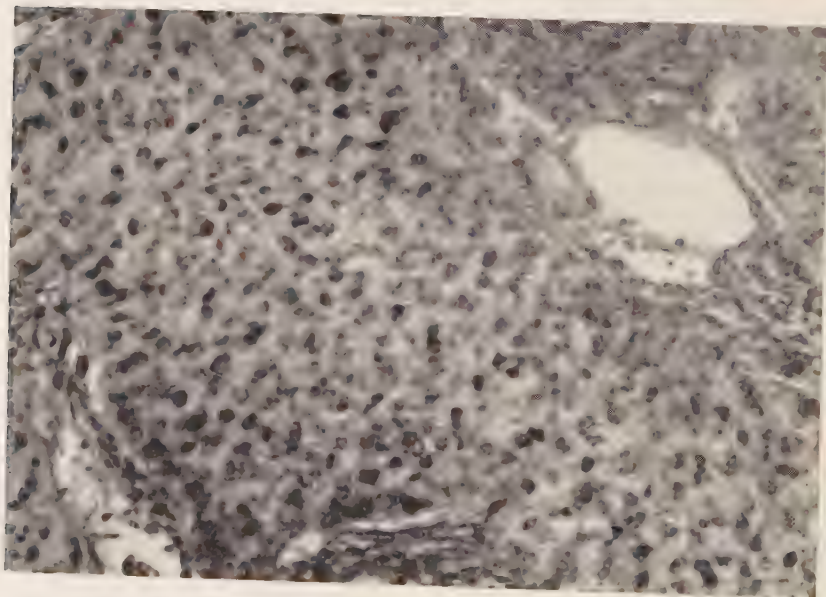


Fig. 12.

Carcinoma of the cervix uteri (No. 224). Biopsy from untreated tumour: Squamous-cell carcinoma without parakeratosis, rather undifferentiated.

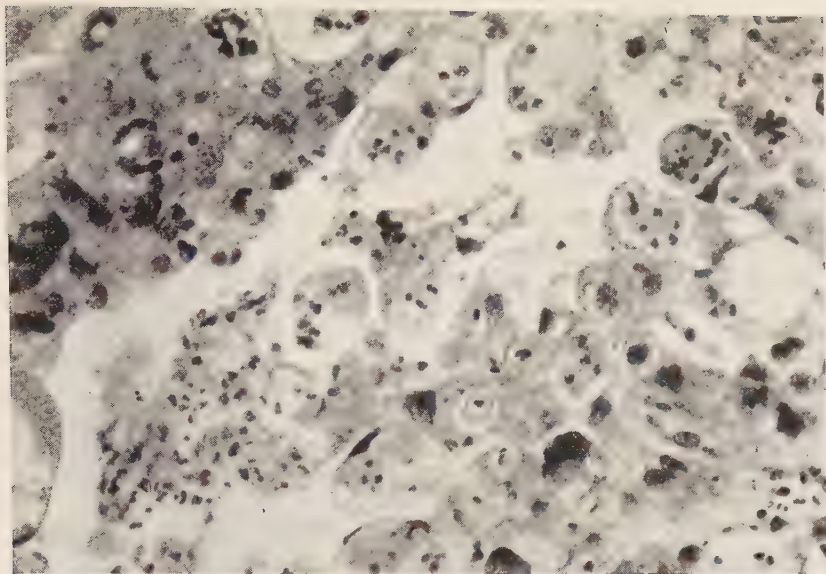


Fig. 43.

The same growth as in Fig. 12. Biopsy after 12000 r. Squamous-cell carcinoma without differentiation, showing violent effects of radiation in the form of degenerated nuclei and a swollen, eosinophilic protoplasm. In addition, numerous leucocytes.

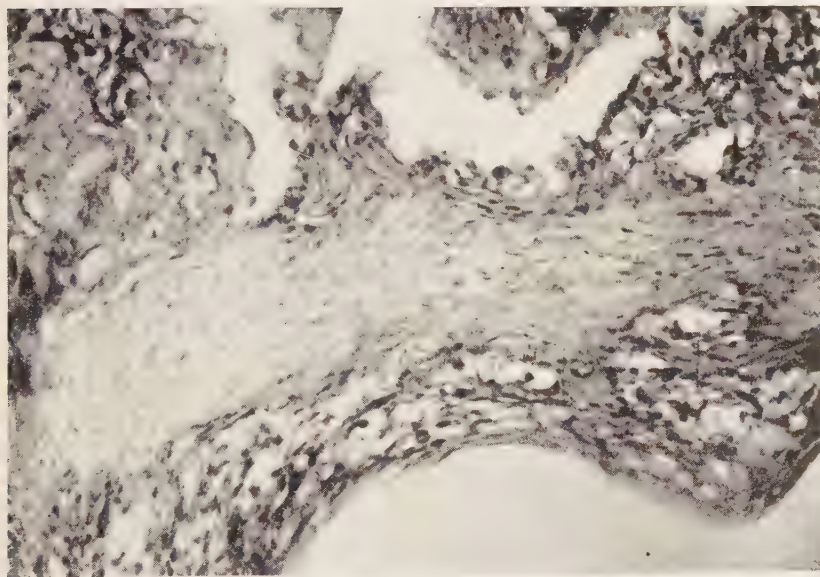


Fig. 44.

Carcinoma of the cervix uteri (No. 234). Biopsy from untreated tumour: Parakeratotic squamous-cell carcinoma. Excessive parakeratosis in the centre of the islets of tumour cells.

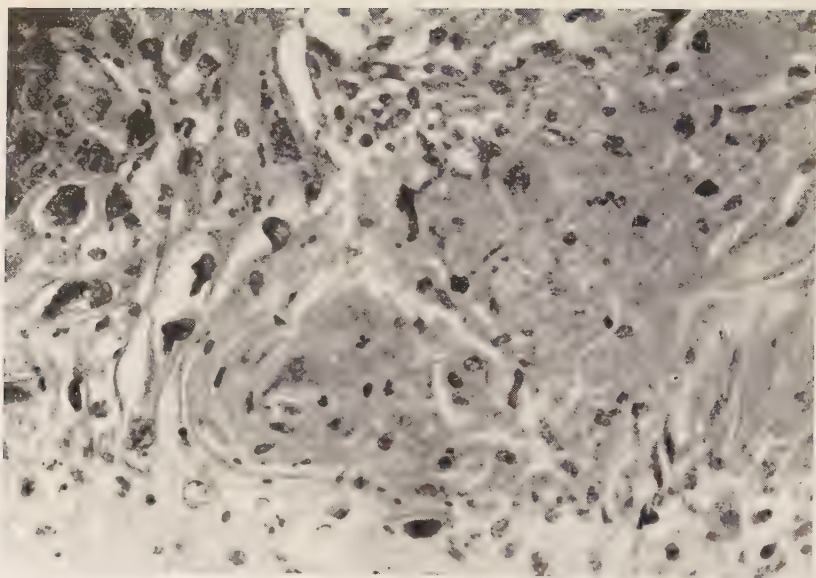


Fig. 15.

Same growth as in Fig. 14. Biopsy after 2000 r: Parakeratotic squamous-cell carcinoma in which the parakeratosis appears to be increasing. Marked effects of radiation.

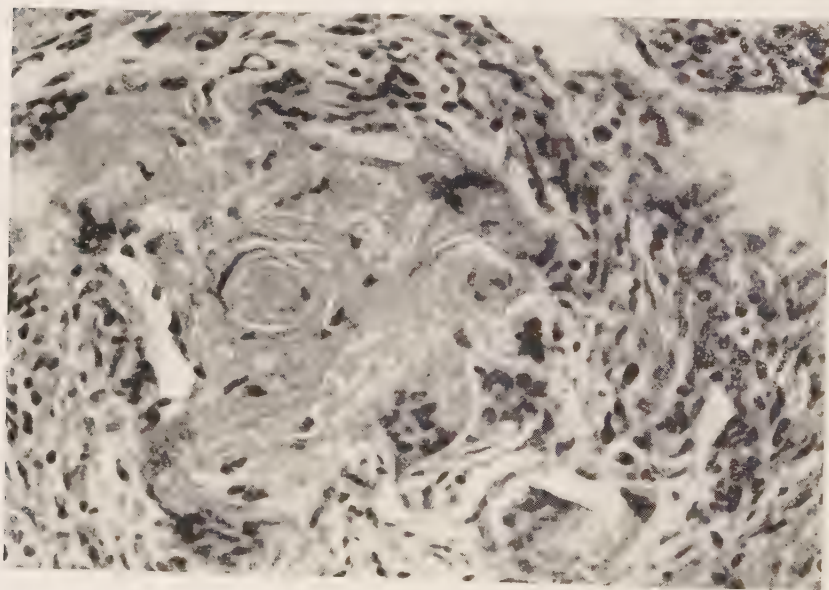


Fig. 16.

The same growth as in Figs. 14—15. Biopsy taken 16 days after the end of X ray therapy and after 1st application of radium, 2 days after 2nd application of radium: Granulation tissue with plaques of a parakeratotic substance which is being absorbed. (Several foreign body giant cells at the bottom of the picture).

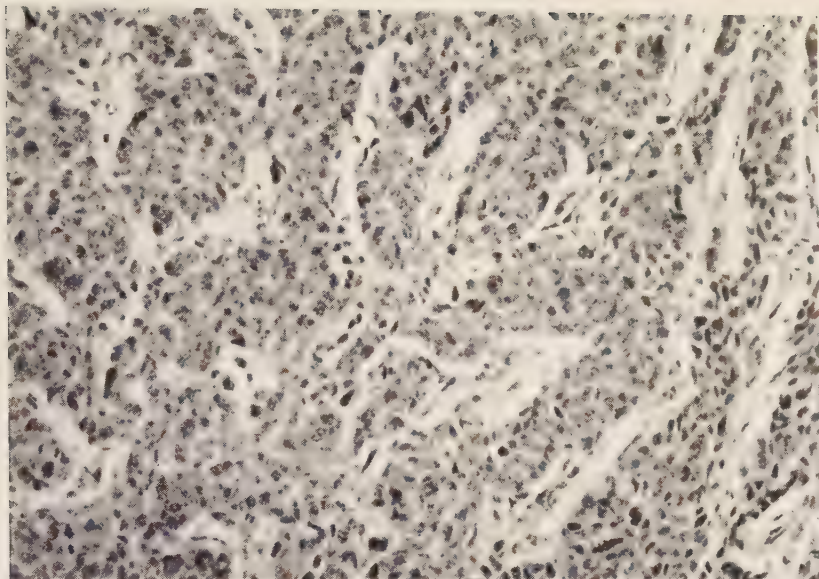


Fig. 47.

Mammary carcinoma Dlb (No. 285, 17137 I), Series 321. Untreated tumour of the tail. A solid, undifferentiated carcinoma with slight polymorphism. No glandular imitations.

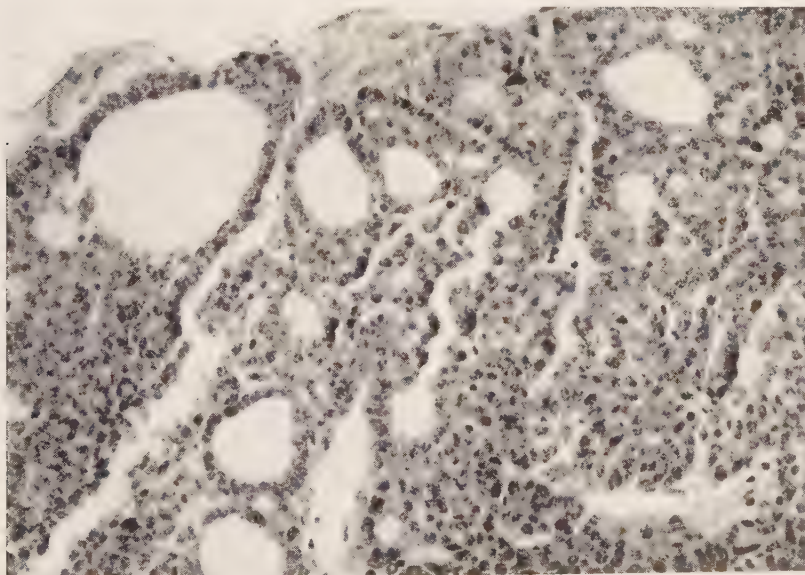


Fig. 48.

Mammary carcinoma Dlb. Untreated intraperitoneal metastasis (17137 III) from the same mouse as in Fig. 17. A predominantly solid carcinoma, showing differentiation in a few places into a definite glandular structure.

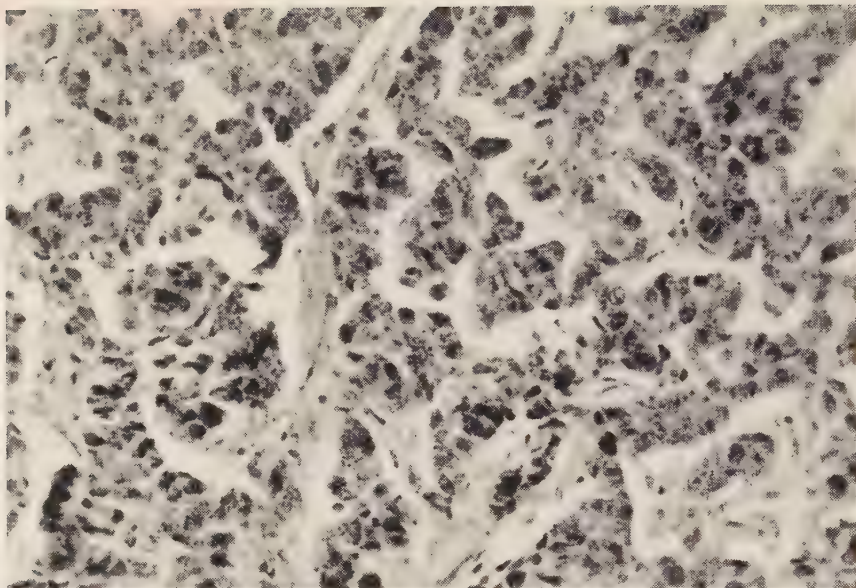


Fig. 19.

Mammary carcinoma Dlb (No. 356), Series 323 a. Killed after $400\text{ r} \times 9 = 3600\text{ r}$. Solid carcinoma without glandular imitations, moderate effects of the radiation in the form of moderate enlargement of the cells and nuclei, hyperchromatism of the nuclei, and numerous pyknotoses. No mitotic figures.

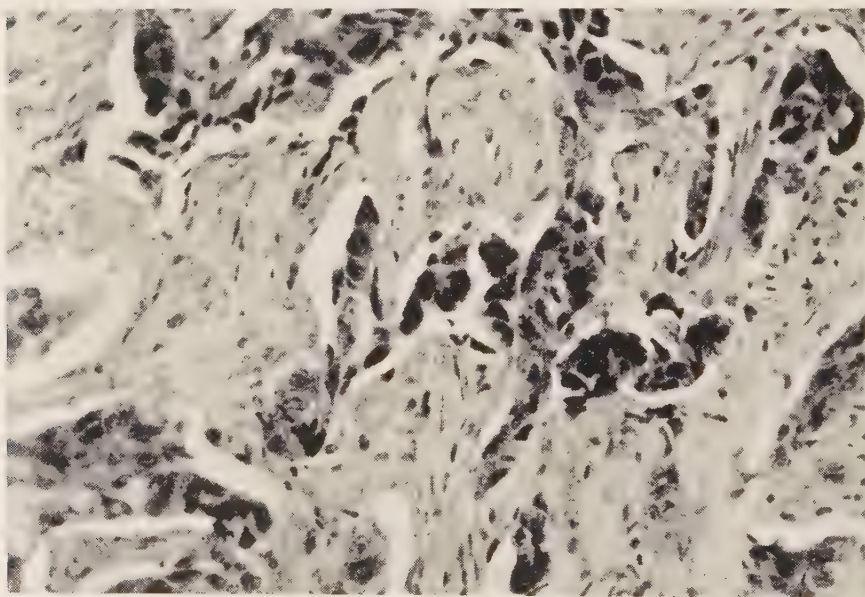


Fig. 20.

Mammary carcinoma Dlb (No. 365), Series 323 a. Killed after $400\text{ r} \times 13 = 5200\text{ r}$. Islets of tumour cells showing marked effects of radiation in a highly sclerosed connective tissue. No glandular imitations. Calcification of the nuclei in a few of the tumour cells in the central area.

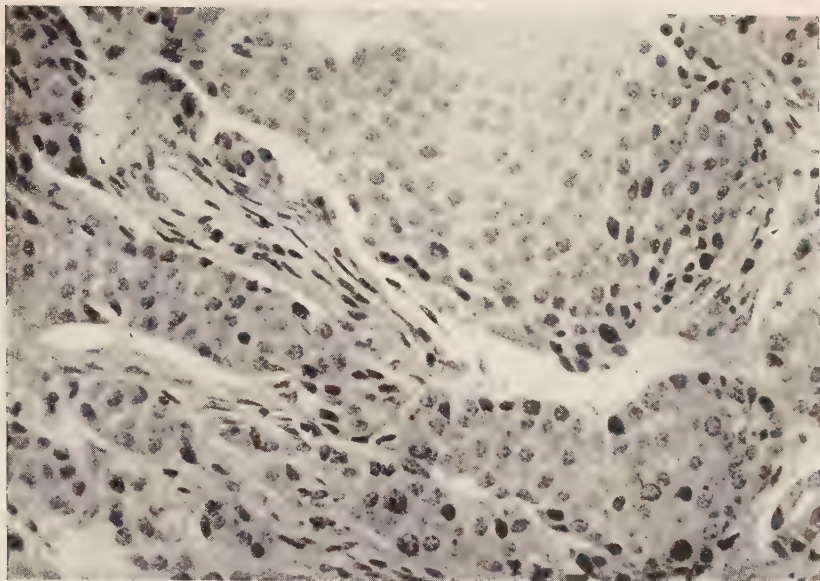


Fig. 21.

Mammary carcinoma (human tumour) (No. 383). Excision biopsy from the untreated tumour: Solid carcinoma. No glandular structures in this section.

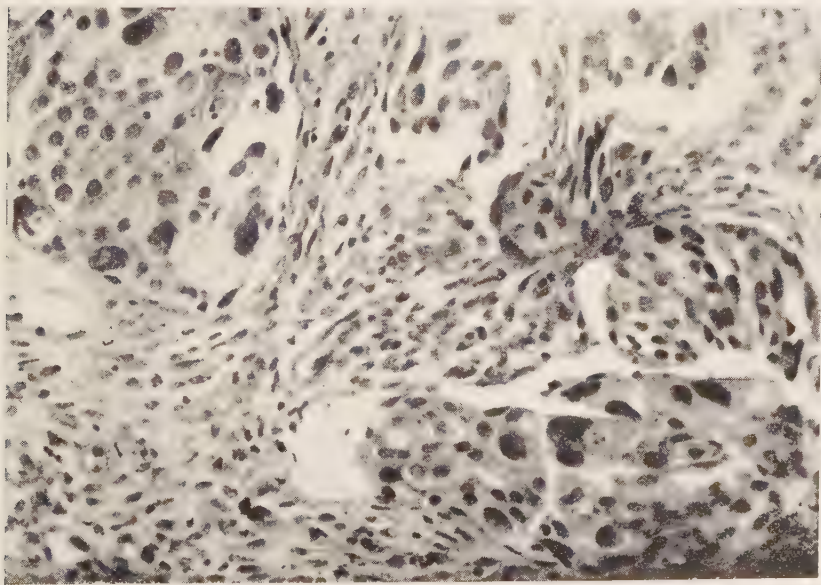


Fig. 22.

The same tumour as in Fig. 21. Excision biopsy after 150 r $\times$ 3-4: Solid carcinoma without glandular imitations, showing moderate effects of the radiation (slight enlargement of the cells and a number of pyknotic nuclei).

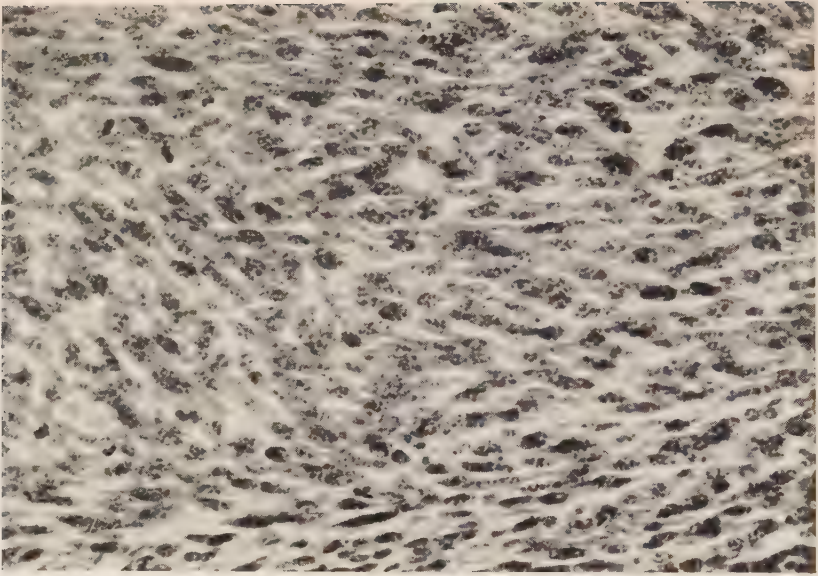


Fig. 23.

Spindle-cell sarcoma Street (No. 416), Series 505. Untreated tumour of the tail: A cellular, undifferentiated spindle-cell sarcoma without fibrils. Moderate polymorphism and a number of mitotic figures.

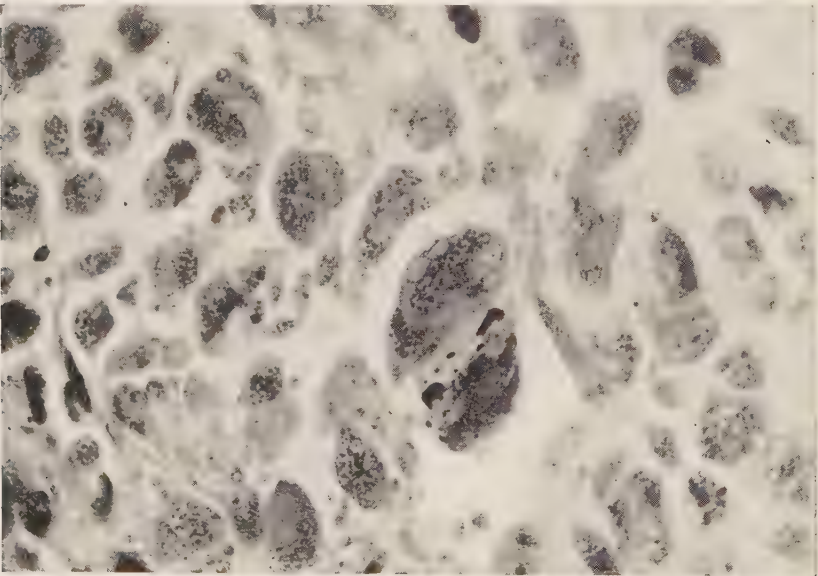


Fig. 24.

Spindle-cell sarcoma Street (No. 511), Series 501—506 a. Killed after fractionated X-radiation, 9 exposures, totalling 4700 r. No differentiation, violent effects of the radiation in the form of monstrous, degenerated, often multiple nuclei in the tumour cell. In the bottom right-hand corner there is an abnormal mitosis. Violent radiation reaction in spite of the radio-resistance of the tumour!

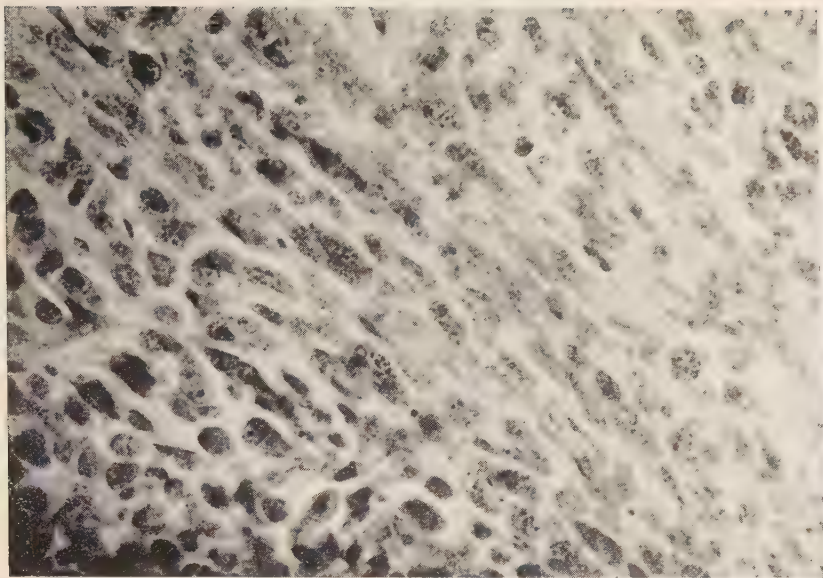


Fig. 25.

Spindle-cell sarcoma Street (No. 512), Series 501—506 a. Killed after fractionated X-radiation, 10 exposures, totalling 5100 r. No differentiation. Marked effects of the radiation. The nuclei are degenerated and the protoplasm blurred. Between the nuclei there are strands of collagen fibrils, derived from the stroma, not from the tumour cells.

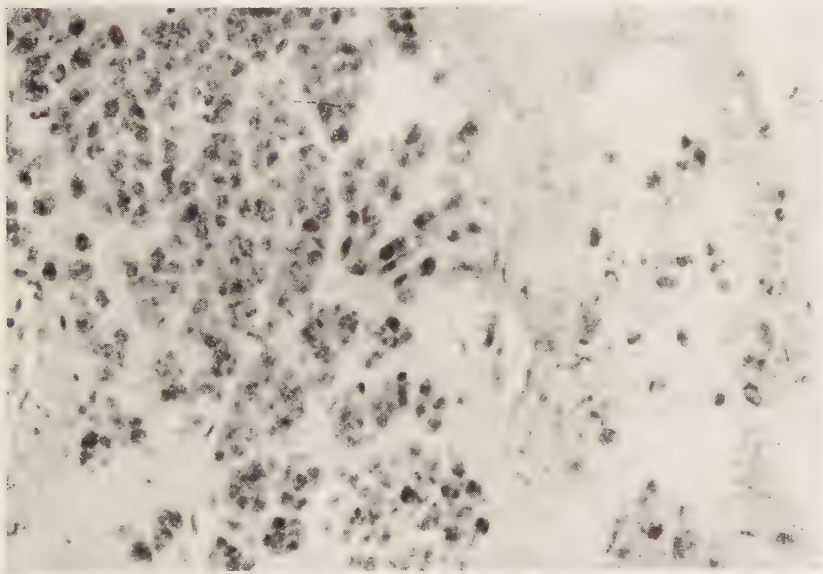


Fig. 26.

Chondrosarcoma (No. 519), Series 442. Untreated tumour of the thigh. On the left typical tumour tissue with densely arranged islets of somewhat polymorphous cartilage cells surrounded by a sparse cartilaginous intercellular substance. On the right a spontaneous necrosis with pyknotic or karyolytic nuclei surrounded by a more abundant, swollen cartilage. No connective-tissue stroma.

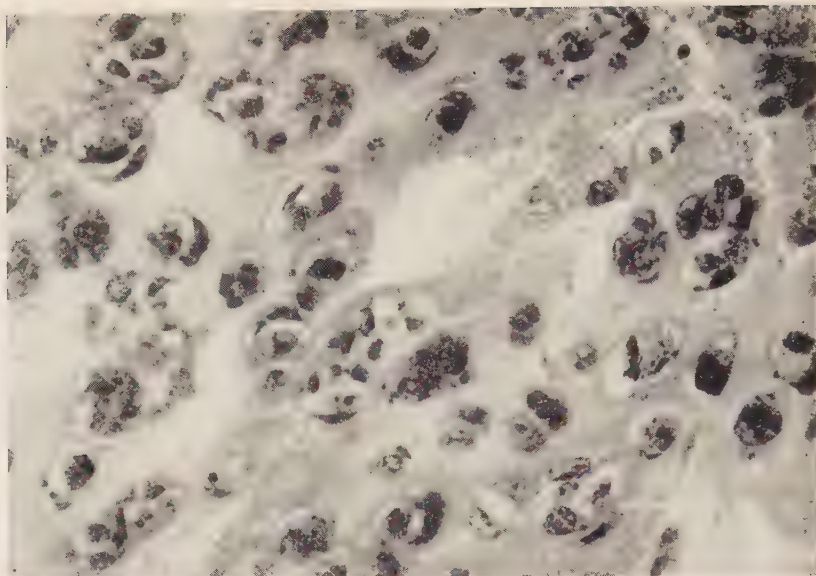


Fig. 27.

Chondrosarcoma (No. 650), Series 444 a. Killed after $400 \text{ r} \times 8 = 3200 \text{ r}$. Marked effects of radiation in the form of vacuolated, degenerated nuclei and a slightly swollen cartilaginous intercellular substance. A few capillaries and small hæmorrhages, but no connective-tissue stroma. Marked effects of the radiation in spite of the radio-resistance of the tumour!

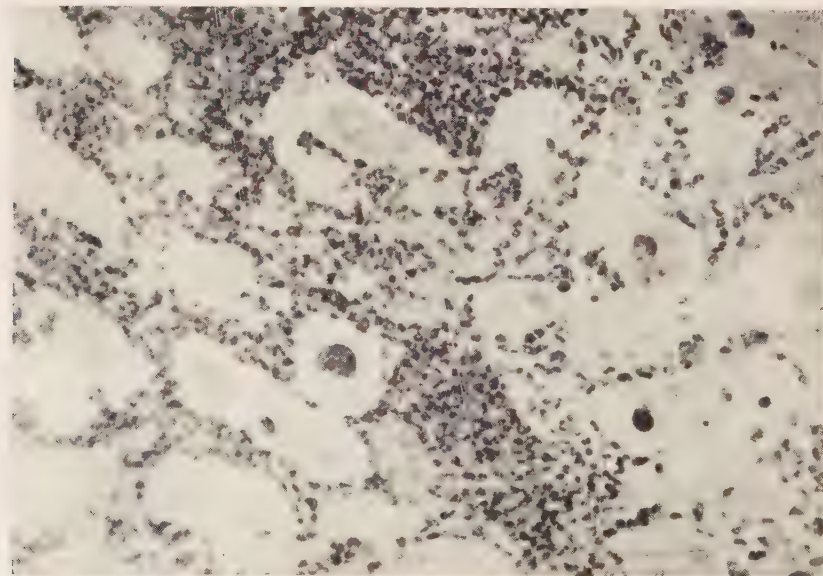


Fig. 28.

Chondrosarcoma (No. 654), Series 442 a. Killed after $400 \text{ r} \times 12 = 4800 \text{ r}$. Violent effects of the radiation in the form of large, partly confluent masses of chromatin fragments. In the mildly swollen cartilaginous intercellular substance there are a few vacuolated nuclei, but no connective-tissue stroma.

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